

Fight against an oppressive bill

British universities have good cause to fight the provisions of the new education bill, rightly last week called oppressive. Will academics have the stomach for what is bound to be a tedious business?

MANY of the difficulties facing British universities would not have arisen if these notionally autonomous and fiercely distinctive institutions had been able to reach a common understanding of the dangers and had been able to agree on a common strategy for their own defence. Over the past decade, too much energy has been spent on protesting at cuts in spending budgets in the British government's exclusive gift, or at decisions such as that requiring universities to charge commercial tuition fees to students from outside the European Communities, too little on turning back threats against the independence of university institutions. Is that about to change? Sir Mark Richmond's speech at last week's *Nature* seminar (see page 291) made a telling case that the British government's bloated Education Reform Bill is indeed a further serious threat of the same kind. Speaking as chairman of the universities' Committee of Vice-Chancellors and Principals (CVCP), he also made a sufficient case for believing that the government's declared objectives could just as well be secured if certain offending clauses in the bill were amended in ways spelled out. If the British university system is to keep its self-respect, those in it should appreciate the vital importance of these seemingly procedural matters.

The central issue is that the bill as drafted would give the Secretary of State for Education and Science the power of detailed regulation of the affairs of individual universities. In principle, he or his successors could require the new Universities Funding Council (due to replace the University Grants Committee (UGC) in the spring of 1989) to make it a condition of continued support for a university that this or that department should be shut, or even that named academics should be fired. Mr Kenneth Baker, the present secretary of state, says he has no intention of using these powers and, less often, that they are powers that he already has. (The second assertion is true only in the narrow sense that UGC's advice on the distribution of funds could be rejected, but only by inviting a serious political row.) Richmond's riposte, in which he seems to have the support of CVCP, is that Baker, if he has no need of these powers, should be willing to see them amended away before the bill becomes law later this summer.

Opposition

If what promises to be the first concerted act of opposition by British universities is to succeed, it is crucial that opposition should be concentrated on a narrow front. It may be that there are even more serious problems in the emerging state of affairs — the continuing shortage of funds (last week's extra in the public expenditure spending budget notwithstanding), the way in which some British universities are being forced for lack of funds and students to abandon the teaching of subjects such as physics, and the way in which able researchers are being stranded by the enforced conversion of their universities into teaching institutions. But there is no consensus among academics on those issues, important though they may be. But last week's seminar showed that, against the drift of recent opinion, there is general agreement that the Education Reform Bill is not, so far as the universities are concerned, a direct threat to their independence, which must be resisted.

The changes Richmond asks for are modest, but reasonable. It will be folly to let the chance of winning them be lost in the search for more ambitious liberation from present discontents.

Questions

Two subsidiary questions are provoked by last week's discussion and by the education bill itself. First, there is the government's proposal to extinguish academic tenure by the cumbersome device of appointing commissioners to rewrite the charters under which British universities operate. Traditionally, tenure has been regarded as the cornerstone of academic freedom, but that is plainly an illusion when there is a real possibility that some universities are unable to pay the salaries of substantial proportions of their staff. It would be ridiculous to resist the rewriting of tenure provisions to allow for the consequences of financial exigencies on the pattern of a university's operations, but it is not fair that academics with unpopular or unorthodox opinions should find themselves first in the firing line. That is why Richmond hopes that the obnoxious education bill will be amended so as explicitly to protect people in such a position, which is reasonable enough. It might be better still to ask that the Privy Council committees that will be asked to do the work should be required to demand of universities, in the new regime, that they should determine which academics lose their jobs when there are difficulties by some deliberate process, explicit enough to be appealed against, in which faculties judge collectively which of their members are the least valuable. If, as is probable, there is now a rapid (and welcome) trend towards diversity in the British system of higher education, only injustice will be done if individuals are all judged by the same hackneyed criteria — the weight of publications, the value of research grants and so on.

The question of the relationship between the universities and the rest of British higher education is also left in limbo. Rubbing a little salt into the universities' wounds, Britain's polytechnics announced their collective welcome for the Education Reform Bill. What they imply, of course, is that they will be happier when supported directly by the central government than through the agency of the local authorities as at present. They may also be banking on the possibility that, in the new regime, they will earn extra brownie points because of their more modest unit costs (probably, but not unambiguously, a result of smaller research overheads). The polytechnics may not yet have read the fine print, for the education bill gives the secretary of state power to shut down particular institutions, or to insist that groups of them should merge. A greater irony is that the structures for both the main sectors of British higher education put forward in the bill provide the simplest way of bridging the present gulf between them — although Baker has apparently no intention of exploiting that state of affairs. But sooner or later, some British government will see the opportunity and will seize it. Then the polytechnics will be howling as the universities are now. For the sake of what the future holds, even the polytechnics should make common cause with the universities on the amendment of the bill. □



Insuring against AIDS

AIDS is a problem for insurance companies, but there are more awkward ethical problems.

THE chief demographic consequence of the epidemic of AIDS (acquired immune deficiency syndrome) is to reverse the tendency in recent years towards declining mortality among those in middle life. That is why, at least in industrialized countries, this novel cause of death has already begun to touch even the uninfected — in their pockets. So much is clear from the response of the life-insurance industry to the spread of AIDS. A document now published by the British Institute of Actuaries shows how the wind is blowing. Even in Britain, where the prevalence of infection by the AIDS virus (HIV) is much less than in, say, the United States, the actuarial calculations of the insurance companies have taken a sombre turn. The 100,000 deaths from AIDS expected by the end of the century (but there may be several times as many) are comparable with the number ordinarily expected among the insured population. The result is that life insurance will cost more.

Life insurance is a distinctively capitalist device by which people seek to avoid the consequences of untimely and unexpected death. The principle is that people in similar circumstances should while alive contribute part of their income into a common pool, with the understanding that the funds accumulated, together with interest (and such profits as accrue on the world's stock markets), are used to compensate the dependants of those among them who die unexpectedly. There is an implied contract binding the members of an insured group that they will not recklessly hazard their lives; professional motor-car racers must pay extra (or be insured separately), as (nowadays) must those who smoke cigarettes. The life-insurance companies administering these schemes are not usually popular; although they are usually tightly regulated to ensure that they do not squander their members' savings, they are frequently accused of making profits at the expense of people's sense of insecurity and of operating over-stringent rules for deciding eligibility for insurance. But few would deny them a place in the modern world.

The arrival of AIDS is bound to upset the insurance companies' apperceptions, not least because it is inevitable that many of those already belonging to insurance schemes are, or will be, infected with HIV. Using a model of the AIDS epidemic which is necessarily uncertain, but which cheerfully makes no allowance for the spread of AIDS to, and subsequently from, women, the Institute of Actuaries calculates that mortality among British males aged between 30 and 40 will be at least doubled, and perhaps multiplied by three or four, in the decade ahead. This result emerges from the conservative assumptions that the proportion of the population at risk of infection (chiefly from male homosexuality and intravenous drug abuse) does not exceed 2.5 per cent of the total of males between 20 and 50 years. The financial consequences are potentially serious for insurers and their customers; the true actuarial cost of life insurance may be two or three times what it has been in the past, perhaps more.

None of this is surprising, nor can people complain if insurance companies seek to insulate themselves from future risks by denying insurance to those at risk of AIDS infection. That is why the companies have been edging towards proposals for carrying questions about sexual proclivities that would previously have seemed impertinent. Blood-tests are also being required for larger contracts. One practical difficulty is that blood-tests cannot spot AIDS infection during the latency period, the duration of which may be long and variable, between infection and the appearance of antibodies. By the same test, the insurance companies cannot hope to tell in advance who will be infected by HIV through blood transfusions.

More serious ethical questions follow. Those applying for life insurance cannot tell at the outset of what is literally a life-long contract, whether their circumstances will change. What, for

example, is to happen to a person who honestly declares himself not to be a male homosexual, but who in later life finds himself drifting into homosexual practices? Under present law, the question having been asked, the insurance company could declare the insurance contract null and void, thus declining to pay insurance benefits to a large proportion of those on their books who eventually die of AIDS. On the face of things, that may seem a just defence of the interests of other policy-holders, but it may nevertheless be thoroughly inequitable. The crucial question is whether the emergence of homosexual tendencies, or even of drug dependence, is to be regarded as a voluntary or an involuntary development. It will take others than actuaries to tell where the truth lies. □

Farming trade wars

Japan should be more forthcoming in its dispute with the United States over farm products.

JAPAN, constantly in hot water with the United States over the frequently ill-founded suggestions that it unfairly skews the balance of trade in its favour by protectionist devices, is behaving tactlessly in the latest squabble — the US demand that trade in agricultural products should be liberalized. The issue has dragged on for years, but came to a head last year when the United States won a ruling from the General Agreement on Tariffs and Trade (GATT) that Japan should liberalize its trade in a dozen different agricultural products, from oranges and milk powder to beef.

The essence of the dispute is that the US government believes that US producers, if on the wrong side of the consumer electronics war, might at least make a better showing with beef and citrus fruit, which is a certainty. The prices of most farm products in Japan are held at levels much higher than world prices — that of rice, for example, is roughly three times the market price. The US case against Japan is nevertheless muted by the common knowledge that the United States is not against farm subsidies as such, and that it differs from most other governments only in the generosity of its support for farmers. The reasons why Japan featherbeds its farmers are largely political, but by ensuring that taxpayers pay above the odds for food, the government has at least found a convenient way of enabling farmers to share in the general prosperity that has come to Japan. That is more respectable than most arguments in favour of agricultural subsidies. In a world in which nobody's hands are clean on agricultural subsidies, Japan is likely to get away with its intended offer at next month's Washington negotiations — the proposal to increase import quotas yet again.

Unfortunately, quotas are not a substitute for free trade, but a poor and partial imitation of it. The immediate beneficiaries of increased quotas are likely to be cattlemen in Texas and orange growers in California, but the beneficiaries of open agriculture would probably be a different lot of people altogether — in the short run, Australian cattlemen, further ahead, closer neighbours of Japan with ambitions to move faster in all-round development. South Korea may already have gone too far down the Japanese road, but there is always China and the rest of Asia.

The Japanese and US governments are not alone in being hostage to their farmers, and to their farmers' votes. Most other governments are also bedevilled by them. But, so far, only the United States has taken an honourable stand by demanding that farm subsidies should be abolished within the framework of the next round of GATT negotiations. What stands out from the Japanese response to US complaints over beef and oranges is that Japan, for one, does not believe that will happen. So much is implicit in the intended offer of larger quotas, not liberalization (which is not inconsistent with import tariffs). That is tactless of a government often pilloried, sometimes wrongly, for seeking to shield its people from competition of the kind by which it has itself prospered. □

Education, Science and Culture gets more in Japan

- Space and nuclear physics to benefit
- Compensation for postdoc exodus

Tokyo

JAPAN'S Ministry of Education, Science and Culture has won some huge increases for its science-related budget in fiscal year 1988. The ministry's requests submitted in September (see *Nature* 329, 187; 1987) were trimmed back during final negotiations with the Ministry of Finance at the end of last year. Nevertheless, the budget is up nearly 20 per cent on last year.

The education ministry's science budget stands in stark contrast to those of the Science and Technology Agency and Ministry of International Trade and

years, the ministry is still slowly proceeding with plans to establish a new national institute for basic research on fusion in Gifu prefecture that will have a helical conductor system as its main facility; ¥50 million is set aside in fiscal 1988 in "preparation funds".

The budget for international exchange leaps by nearly ¥5,000 million (\$40 million). Japan has recently come under criticism for sending too many scientists each year to the United States, France, West Germany and the United Kingdom, with too little traffic the other way. The

number of domestic post-doctoral fellowships is similarly increased by 80, with 404 new 2-year fellowships in fiscal 1988. But the post-docs may be envious of their Western colleagues — while they have to survive on ¥717,000 a month (equivalent to about \$20,000 a year) the overseas visitors get close to \$30,000 a year, tax free.

The budget for marine research has swollen by an order of magnitude compared with that of 1986, but all the extra money is going towards construction of a

new research vessel for Tokyo University.

Several thousand million extra yen are also being ploughed into the development of information networks in the national universities and into promotion of joint university/industry research. But by far the largest budgetary increase is for space research. The ministry's Institute of Space and Astronautical Sciences gets a massive boost of ¥8,000 million (about \$60 million) over last year. In part the increase can be explained by the fact that the institute did not launch any satellites in the present fiscal year. Nevertheless, the budget is by far the highest ever.

The institute has had a string of major successes over the past few years — the Halley's comet probes in 1986 and the X-ray satellite *Ginga* which detected X-rays from the supernova in the Magellanic Cloud at the end of last year (see *Nature* 330, 230; 1987). And the institute is now angling for a bigger rocket to send probes to the planets. But the extra funds in 1988 are nearly all for satellite programmes which have already been approved and use existing rocketry. **David Swinbanks**

SSC shortlist now cut down to seven sites

Washington

THE US Department of Energy (DoE) has officially accepted the shortlist of sites for the Superconducting Supercollider (SSC) that was recommended by the National Academy of Sciences (see *Nature* 331, 2; 1988). But the final list has only seven sites — one each in Texas, Illinois, Arizona, Colorado, Michigan, Tennessee and North Carolina — because New York Governor Mario Cuomo asked DoE to remove from further consideration the state's proposal to build the SSC near Rochester.

Cuomo was responding to the wishes of local residents and congressmen, who are in opposition to disruption of the rural area, and takes New York, which originally had four prospective sites, out of the running.

New York's bid for SSC, led chiefly by Lieutenant Governor Stan Lundine, has been ill-starred. One site was rejected because it crossed into Canada, and another was withdrawn earlier after a noisy town meeting. Cuomo asked DoE to reconsider the fourth New York site, which did not make the shortlist, but DoE was evidently unwilling to tinker with the academy's selection and risk protests from the many other states that were left out. In any case, Cuomo has never made any strong public expression of support for SSC, and did not press DoE hard on New York's behalf.

Unlike New York, California officials had been keen to host the SSC, and had pushed hard but unsuccessfully to be added to the DoE shortlist after the academy committee passed it over. Thomas Everhart, president of the California Institute of Technology and a member of the academy selection committee, said the committee felt that the proposed California site, near Stockton, had a number of problems, not least of which was its distance from a major international airport.

The committee felt SSC should be located where it can be easily reached by scientists from around the world. After a site was determined to be acceptable in geological terms, ease of access and regional resources weighed heavily in the committee's deliberations.

Everhart said the Illinois site near Chicago, and the Texas site near Dallas, both provide excellent travel facilities. The committee was concerned about the question of local opposition, but did not include this in the decision process.

David Lindley & Joseph Palca

Science Budget for Ministry of Education, Science and Culture

	1986	1987	1988	(% change from 1987)
Grants-in-aid of research	43.5	45.1	48.9	(+ 8.4)
Maintenance of scientific research system	NA*	NA	1.7	
Promotion of government industry research	6.8	7.9	8.9	(+12.6)
Research fellowships	0.8	1.1	1.5	(+30.1)
Nuclear fusion	8.2	7.7	7.6	(- 1.2)
Accelerator physics (TRISTAN)	13.0	12.9	16.5	(+28.2)
Space science	12.4	11.8	19.8	(+67.4)
Marine science	0.5	3.0	5.5	(+84.0)
Earth science	2.0	2.1	2.1	(+ 1.0)
Antarctic research	3.1	2.9	2.8	(- 1.7)
Cancer research	1.8	1.8	2.2	(+19.9)
Information networks	NA	17.2	19.0	(+10.1)
International academic exchange	NA	5.5	6.6	(+19.8)
International student exchange	11.7	14.5	18.3	(+26.0)
Japanese language education	0.2	0.3	0.3	(+ 1.5)

*NA: equivalent figure not available

Industry which were held to meagre increases despite an expansionary budget for fiscal 1988 (see *Nature* 331, 198; 1988).

Grants-in-aid for scientific research are boosted by nearly ¥4 thousand million (\$30 million). Half of the increase goes to grants in the category "priority areas of research", which supports projects involving large numbers of scientists from all over Japan. For example, several hundred million yen will go to a 3-year investigation of the new high-temperature superconductors by a team led by Professor Muto of Tohoku University, who has links with Shoji Tanaka's group at Tokyo University. Tanaka also received a large grant for research on the new superconductors from the ministry this fiscal year.

TRISTAN, the world's largest electron-positron collider, which began full-scale experiments in May, gets several thousand million yen extra in fiscal 1988 to increase operating hours and to boost power through the emplacement of superconducting radio-frequency cavities. And, although funds for fusion research have steadily declined over the past 3

New impetus for French AIDS health education campaign

Paris

FACED with a projected 10,000–15,000 declared cases of AIDS (acquired immune deficiency syndrome) in 1989, France is to step up its health-education campaign and provide more facilities for the care of AIDS sufferers. During a cabinet briefing on 20 January, Health Minister Michèle Barzach told ministers that FF930 million (\$165 million) had been set aside in 1988 for prevention, education and health care. Barzach also reported on the success of the existing measures (see *Nature* 328, 3; 1987) and announced new initiatives.

At the end of last year, 3,073 cases of AIDS had been recorded in France, including its overseas territories, of whom 45 per cent had already died. Known cases were in the Paris region (58 per cent) and southern France, around Marseilles, Toulon and Nice. Barzach put at 150,000–200,000 the number of individuals infected by the human immunodeficiency virus (HIV), but not showing symptoms of AIDS.

Among the public health measures undertaken so far, Barzach mentioned the mandatory, anonymous notification of all AIDS sufferers; the availability to all of anonymous screening; the systematic screening of donors of organs or sperm; the establishment of 11 information and care centres; the signing of a legal agreement between the US Department of Health and Human Services and the Institut Pasteur ending the patents dispute

over the antibody test; the deregulation of public advertising of condoms; and the availability, without prescription, of hypodermic syringes. In addition, the antiviral agent AZT is to be released into the market.

Since the start of the campaign, there has been a 38 per cent increase in sales of condoms in pharmacies and a marked increase in demand for screening tests. In 1987, 2.3 million ELISA (enzyme-linked immunosorbent assay) tests were sold in France, compared with 800,000 in 1986. Encouraged by these "very positive" results, Barzach noted that each French department would have a free screening centre this year.

In addition to the 11 existing AIDS clinics and advice centres, a further 11 are to be opened, two of them in overseas departments, French Guiana and the Antilles. Barzach also aims to ask general practitioners to play a bigger role by encouraging patients to be screened at 'key moments' in life, such as marriage and pregnancy.

Peter Coles
● Ten haemophiliacs suffering from AIDS have joined together in a legal fight for compensation against the French national blood transfusion service (CNTS). They claim that CNTS continued to supply contaminated blood for "several months" during 1985, even though treated imported supplies from other European countries and the United States were available. □

Bavarian government maintains reputation for AIDS controversy

Munich

THE appointment of Michael Koch, a Swedish physician, by the Bavarian Interior Ministry on 1 January to develop publicity material concerning AIDS (acquired immune deficiency syndrome) has precipitated a row in the West German Bundestag.

The Social Democratic Party (SPD) claimed that Koch's testimony before a Bundestag panel in September was "a mixture of 80 per cent irresponsible and unbelievable claims and 20 per cent scientifically sound views". Koch's appointment was also condemned by members of the Green Party and of the Christian Democrats (CDU).

Koch is to receive about DM100,000 in 1988 for his work. He is expected to pro-

duce easy-to-understand pamphlets about AIDS for health officials. Bavarian Interior Ministry spokesman Christoph Hillenbrand vigorously denied that Koch would serve as a policy adviser.

Koch was accused by his opponents of calling for the internment of carriers of the human immunodeficiency virus (HIV) who do not behave "reasonably", such as prostitutes who do not use condoms. Hillenbrand pointed out that Koch would advise such measures "only as a last resort". But "isolation" would be in keeping with the federal law on epidemics, said Hillenbrand.

Bavaria has been a source of controversy over AIDS since early last year, when it began to test immigrants from outside the European Community for HIV and to refuse entry to carriers. The Bavarian AIDS programme has been rejected by West German Health Minister Rita Süßmuth (CDU). Steven Dickman

US nuclear waste site "unsuitable"

Las Vegas, Nevada

NEVADA Governor Richard Bryan claimed on 21 January that the Department of Energy (DoE) intentionally suppressed a study by a government scientist that raises serious questions about the suitability of Yucca Mountain as the site for the nation's first high-level nuclear waste repository.

Bryan said the study of water flows in the Death Valley system by DoE scientist Jerry S. Szymanski was given to the Energy Department in November, a month before Congress chose Yucca Mountain, 80 miles north-west of Las Vegas, as the national nuclear repository.

Szymanski said that underground pressure from volcanic and seismic activity may force corrosive water within the mountain's tuff through cracks and fissures, eventually leading into the repository where nuclear containers could corrode.

Szymanski, the governor said during a press conference, expressed concern to DoE superiors as early as 1984. Carl Gertz, Nevada Nuclear Waste Project Manager said DoE asked Szymanski to prepare his report in November last year, knowing how he felt about Yucca Mountain. Gertz said he received the report on 22 December.

Szymanski's report recommends a thorough review of 10 years of data concerning water flow at Yucca Mountain, complete investigation of rocks marked by water and chemical analysis of the water in the rocks and soil. Mary Manning

Second AIDS trial

Washington

THE US National Institutes of Health (NIH) and the biotechnology company MicroGeneSys have announced another clinical trial of their jointly developed vaccine against AIDS. The vaccine, which consists of the gp160 envelope protein from HIV, the virus causing AIDS, is to be administered to 72 healthy seronegative male and female volunteers at six federally sponsored Vaccine Evaluation Units.

The first clinical trial of the vaccine that began in October involving homosexual men will continue. But so far only 31 volunteers out of the 81 required have been found (see *Nature* 327, 687; 1987).

The six Vaccine Evaluation Units — at Baylor College of Medicine in Houston, Texas; Johns Hopkins University and the University of Maryland in Baltimore, Maryland; Vanderbilt University in Nashville, Tennessee; Marshall University in Huntington, West Virginia; and the University of Rochester in Rochester, New York — will recruit volunteers locally. Two test groups will receive differing dosages of the actual vaccine, and control groups will receive either recombinant hepatitis B vaccine or the adjuvant alum. Carol Ezzell

Correction: French research

There are 270 posts available at CNRS in 1988, of which 150 are new posts (see *Nature* 331, 200; 1988). □

A threat to "the very heart" of academic freedom

London

THAT academics of all political persuasion seem to be united in their opposition to the Education Reform Bill was made clear at a seminar last week, organized by *Nature* and attended by nearly 100 politicians and leading academics. Two principal conclusions emerged: first, that there is an overwhelming degree of consensus on the issues, and that many of the government's keenest supporters are incensed by the proposals; and second, that the universities need to learn quickly how to win new friends and influence people, something that they have woefully neglected to do in the past.

The bill is not much concerned with higher education, but the few short clauses relating to universities have far-reaching implications, opening the way for direct political intervention in the management of individual departments within institutions. Professor Sir Mark Richmond, vice-chancellor of the University of Manchester and chairman of the Committee of Vice-Chancellors and Principals (CVCP), told the seminar that the bill, if enacted, would "strike at the very heart of academic freedom".

The bill proposes to create new funding bodies for the university and polytechnic sectors. The creation of a Universities Funding Council (UFC) to replace the University Grants Committee (UGC) is not in itself contentious. What have horrified the academic community are clauses in the bill that would establish a 'chain of command', with the secretary of state able to give specific instructions to the funding councils which would impose conditions on institutions. Failure of a university to comply with UFC's directions would require the repayment, with interest, of any grants received through the UFC.

Richmond argues that if the aim of the bill, as far as it affects universities, is to create a system under which institutions can be forced to adapt to economic circumstances, then it is misguided. "Change is already happening. The legislation designed to bring about this change actually threatens to slow its progress. It will hinder. It will hamper. It could prove to be a serious constraint to those who want to manage well and responsibly."

Further, a mechanism that allows ministerial intervention (through conditions imposed on the funding council) in an individual department of a university threatens three fundamental freedoms: "the freedom to research in subjects of as yet unrecognized importance, the freedom to question received wisdom; and the freedom to be protected from direct and narrow political interference by the government of the day."

That the universities are capable them-

selves of instigating change is self-evident, says Richmond. Since the cuts of 1980-81, teaching staff has fallen by 12 per cent. Student numbers were at an all-time high of 301,000 by 1986-87. Numbers of part-time students and short courses, many run for industry, have also proliferated. Total research income since 1981-82 is up by 85.7 per cent, with the rate of increase of earnings from industry and commerce outstripping that from the research councils. Overall, the proportion of income from block grants is now 57 per cent, compared with 77 per cent a decade ago. Consultancy and applied research activities hardly existed before 1980, says Richmond. Now around 100 university companies provide such services, with more than 500 spin-off companies.



Sir Mark Richmond will discuss his view that "change is already happening" in a forthcoming *Commentary*.

The proposals contained in the bill would hinder an institution's ability to innovate, he says. Clause 94(3) reads: "The Secretary of State may make grants to each of the funding councils of such amounts and subject to such conditions as he may determine"; and clause 94(4): "In exercising their function . . . each of the funding councils shall comply with any directions given to them by the Secretary of State". Clause 92(6) reads: "The [funding] councils shall have power to make payments subject to such terms and conditions as they think fit". A later clause enables the council to reclaim any funds, with interest, if conditions are not met.

The possibility of secondary legislation is also causing concern. Last summer, the Department of Education and Science (DES) produced a consultation document which proposed that allocation of money from the funding councils would be in the form of contracts — leaving open the possibility that an individual university's funding would comprise a large number of specific 'mini-contracts', precluding the possibility of switching funds from one to another. Although the bill does not contain the word 'contract', it does refer to "payments subject to terms and conditions".

Richmond denies the government's claim that the proposed system, because it

will be defined in law, will be clearer and more limited than the all-embracing power presently resting with the secretary of state. (The UGC is effectively a sub-committee of the DES.) At present, the UGC advises the government on the block grant to be paid to each university, which is handed over in one lump sum, to be spent as the university thinks best — subject to scrutiny by its own auditors and the National Audit Office. The UGC can offer 'advice', which can be ignored, on the understanding that such action could lead to grant cuts in future, "but very different from a detailed and binding instruction, backed with the ability to demand repayment of grant with interest, which this bill would allow".

Richmond decries the notion that cost-effectiveness should be the criterion by which to manage a university. Universities, he says, are in part businesses whose product is ideas, and that as such it would be inappropriate for politicians or civil servants to set themselves up as commissioning agents. "Who, in Whitehall, or indeed anywhere else, would for example have written a contract in advance for one A. Einstein to produce the research published in the *Annalen der Physik* in 1905 under the title *A new determination of molecular dimensions*?"

The degree of concern expressed at the seminar was typified by Professor Henry Chadwick, master of Peterhouse, Cambridge. About clause 94(1), which allows the secretary of state to confer additional functions on the funding council "as he thinks fit", he said: "I am alarmed that the bill confers powers on the secretary of state to tell scientists and scholars precisely what to do. He only has to get it wrong once for the brain drain to reach an unprecedented scale." The clause is, he said, "insufferable in a free democratic society".

Sir Patrick Neill, vice-chancellor of the University of Oxford and vice-chairman of the CVCP, says amendments are being drafted to curb the secretary of state's powers of direct intervention. Lord Flowers, vice-chancellor of the University of London, suspected that the offending clauses were the work of civil servants "who have an eye on the future".

It was for Labour peer Lady White and the Liberal leader in the House of Lords, Lady Seear, to offer the practical advice to lobby local members of parliament, particularly Conservative back-benchers, and in the Lords to focus on cross-bench peers. There was little dissent from the view of Professor Norman Saunders, of the University of Southampton, that the price is now being paid for the failure of the universities to raise their public profile to demonstrate their integral role in the nation's culture.

Simon Hadlington

New information throws light on how Yuri Gagarin died

London

LAST week, *Pravda* published what appears to be the final verdict on the death of Yuri Gagarin, the first man in space. Gagarin and his co-pilot, Vladimir Seregin, died in an air crash in March 1968. The findings of the official inquiry were not, however, published at the time, and a partial account in *Pravda* last year concentrated on Gagarin's state of health before and during his last flight, and his heroic final efforts to wrestle the aircraft out of a near-vertical dive, leaving open the question of what had caused the dive.

The original silence had inevitably generated many rumours ranging from Gagarin being drunk at the controls to his having fallen from political favour and eliminated in a staged "accident". Last year's *Pravda* article, co-signed by a leading astronautics expert, Professor Sergei Belotserkovskii, and cosmonaut Aleksei Leonov, effectively dispelled these rumours. But the lack of a clear-cut expla-

nation for the accident generated the rumours that there had been some kind of cover-up. During the past ten months, *Pravda* received many letters asking for more details. Last week, therefore, Belotserkovskii provided a further explanation.

According to this new reconstruction, Gagarin and Seregin were in a Mig-15 jet coming in to land from an altitude of 4,200 m and were between two layers of cloud, with no visibility of the horizon. The flight controller had informed them that the height of the lower layer was 900 m; it was in fact 400 – 500 m. (The controller's figure was correct, but for the vicinity of the airfield, not for the flight zone.) As they were making their approach, a Mig-21 jet, far larger than the Mig-15, passed within some 500 m of Gagarin's aircraft. The Mig-21 pilot apparently never saw the Mig-15 or knew that it was nearby. The smaller aircraft, however, was hit by the jet-wash, and

began to dive. On the basis of the erroneous information, Gagarin and Seregin, who were still in the clouds, believed they had enough height to pull out of the dive. They were wrong — by a mere 250 m, or 2 seconds.

This new scenario, Belotserkovskii stressed, has been established during the past ten months, using meteorological records, voice tapes computer simulations, and "personal archive materials". (Black box flight recorders, he explained, were not installed in Mig-15s at that time — a surprising revelation.) The fact that so much new information could be established almost 20 years after the accident, he explained, was not because of an earlier cover-up but to the fact that although the original commission of inquiry carried out "careful and profound investigations in all directions", the commission "did not carry its work through to the end". The two sub-commissions into which it was divided did not coordinate their work properly, he said. Most important, there was no "group of competent experts" that could have studied and appraised all the data presented by the working groups, and drawn general conclusions from them. This, if *glasnost* continues, may evoke a further deluge of questions: why not? **Vera Rich**

US telescope plans postponed until accuracy demands are met

Austin, Texas

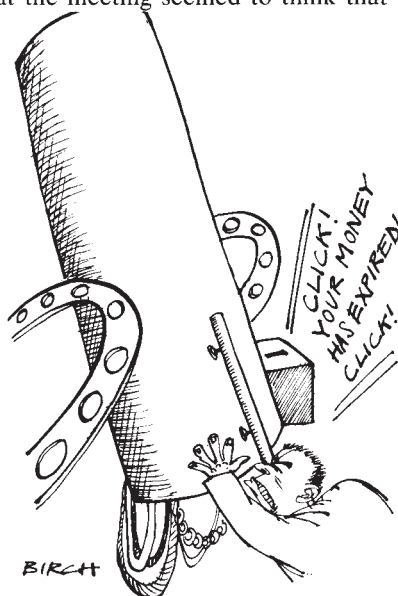
FACED with uncertain finances and some technical doubts, the National Optical Astronomy Observatory (NOAO) will not pursue, for the time being, plans to build the National New Technology Telescope (NNTT), a giant four-in-one interferometric array of mirrors. NOAO, which operates the Kitt Peak Observatory in Arizona and the Cerro Tololo Observatory in Chile, hopes instead to submit a proposal before the end of this year to build two 8-m telescopes, one on Mauna Kea, Hawaii, and the other somewhere in the Southern Hemisphere.

One participant in the decision said that it was a consequence of "realistic thinking" about the likelihood that the National Science Foundation (NSF) would provide funds for NNTT before it had been demonstrated that large mirrors of the required accuracy could be made.

Speaking here at this month's American Astronomical Society meeting, Sidney Wolff, director of NOAO, also had a wider caution for astronomers. After years of essentially level funding from NSF, most programmes had been trimmed to the bone, and the only alternative to a general "slide into mediocrity" might be to close down some facilities. No such decision has yet been made, but Wolff made it clear that she saw the habitual defence by all astronomers of all NOAO activities as a questionable strategy for the

near future.

NOAO is expected to play a double role, providing astronomical facilities for general use but also leading the development of new techniques. Some observers at the meeting seemed to think that the



second role could be reduced or even abandoned, but Wolff was clearly unprepared to allow superiority to fall into the hands of the European Space Agency, which intends to go ahead with its own version of NNTT (see *Nature* 330, 593; 1987). **David Lindley**

India modernizes crematoria

New Delhi

WITH the Hindu custom of burning dead bodies in funeral pyres threatened by rising fuelwood prices due to rapid depletion of forests, the Indian government is seriously thinking of modernizing its crematoria.

Apart from cooking, fuelwood in India is used chiefly for cremation. A massive programme is already under way to introduce smokeless, fuel-efficient "chulas" (wood-stoves) in villages. And now the government has launched a scheme to install new designs of wood-burning crematoria twice as efficient as the existing ones.

Some 1,500 such specially designed crematoria have been installed in the state of Gujarat during the past five years under a project supported by the World Bank. They have shown a 50 per cent saving in firewood and a halving of the previous cremation time of three hours. The Energy Advisory Board recommends replacing all existing crematoria by the end of 1990.

In Delhi alone, some 50,000 bodies are burned each year in 12 crematoria with 260 platforms, one cremation consuming a six months supply of cooking fuel for a family. The high cost of wood means the poor are forced to throw the dead bodies into rivers after partial burning. Along the banks of the Ganges, where floating bodies are common, the government is setting up 29 electric crematoria, eliminating the need for wood.

K.S. Jayaraman

West Germany's nuclear-fuel transport scandal has long half-life

Munich

THE latest — and largest — in a series of scandals involving the nuclear industry continues to reverberate throughout West Germany. The scandal, now being called "Urangate" by some, has grown to such proportions that it casts a long shadow over the future of the West German nuclear programme.

In a rare show of interparty unity, the Social Democrat Party (SDP) joined the governing centre-right coalition in the Bundestag (parliament) to create a committee that will investigate charges of bribery and irregularities made against the nuclear transport company Transnuklear (see *Nature* 331, 6 and 331, 106; 1988). The committee, which has the legal power to prosecute, was formed after weeks of prodding by the environmentalist Greens.

The committee's first task will be to investigate rumours that weapons-grade nuclear material from West Germany has gone via Belgium to Libya and Pakistan in violation of the international nuclear non-proliferation treaty. The rumour has been denied by all three countries.

One reason that the rumour caused such consternation was that it appeared to explain the huge sums (DM21 million) involved in the bribery scandal. The reason for the size of the payments is perhaps the most intriguing missing piece to the Transnuklear bribery puzzle.

The scandal has already triggered protests. Police broke up on 22 January a five-day blockade of a federal storage site in Lübeck where 21 tonnes of uranium hexafluoride were waiting to go to Sweden.

Uranium hexafluoride is a raw material

for nuclear fuel elements. A group of about 50 farmers blocked the gates to the temporary nuclear waste storage site at Gorleben for a day on 22 January to protest against the illegal storage, revealed earlier in the week, of barrels of waste which were starting to break open.

The scandal has given a big boost to the anti-nuclear Greens — a "qualitative leap" according to Green Bundestag member Otto Schily, the only Green member of the investigation committee.

The complete West German nuclear programme will depend on transport of fuel elements between regular nuclear power stations, breeder reactors and reprocessing plants. But the impact of the scandal may be sufficient to halt further construction on the as yet inoperative fast breeder at Kalkar and reprocessing plant at Wackersdorf, each the first of its kind in West Germany. The Bundestag committee is expected to produce its final report by the end of 1989.

Steven Dickman

● The experimental breeder reactor at Kalkar is facing new difficulties. The plant's principal owner, Schnellbrüter-Kernkraftwerksgesellschaft (SBK), surprisingly withdrew on 11 January its application for a licence to load fuel elements into the still incomplete reactor. Instead, SBK is asking for approval to make a variety of safety improvements at the plant. Licensing authorities from the West German of North Rhine-Westphalia see the move as a ploy to gain the "overall approval" that the authorities have consistently withheld (see *Nature* 326, 534; 1987). Construction costs for the 300-MW plant have exceeded DM 7,000 million. □

Synchrotron budget

THE European Synchrotron Radiation Facility has agreed on a budget for 1988 of 108 million French francs, leaving open the extent of British participation in the seven-year project. Construction is to begin this year in Grenoble, France, on the synchrotron, a joint project of ten European countries. The United Kingdom is willing to provide no more than 10 per cent of the funding, but West Germany and France are insisting on a higher UK contribution (see *Nature* 330, 513; 1987). The facility should be completed by 1994. S.D.

Superconductor plans

A THREE-YEAR, £16-million national programme of superconductivity research has been announced by Britain's Department of Trade and Industry. The programme is one of the first manifestations of the government's recently declared intention to stimulate research collaboration between private companies. Successful proposals will receive half their funds from the DTI, which has earmarked £8 million for the scheme. S.L.H.

More on R&D in UK

A SURVEY of industrial expenditure on research and development in Britain in 1986, published last week by the Department of Trade and Industry, shows an increase in spending of about 10 per cent compared with 1985, to £5,700 million, a rise in real terms, after allowing for higher costs, of about 7 per cent. The proportion of funds provided by the government remained static, at 23 per cent, while industry funding fell from 66 per cent in 1985 to 64 per cent in 1986. The amount of funds from overseas increased from 11 per cent to 13 per cent. Between 1985 and 1986 the total number of people employed in industrial research and development increased by only 1,000, to 174,000, compared with 195,000 in 1981. S.L.H.

King Faisal prizes

PROFESSOR Janet Rowley, of the University of Chicago, is co-winner of this year's King Faisal International Prize in the field of medicine. She is the first woman to receive a King Faisal Prize since the five awards, worth £60,000 each, were introduced in 1983. She shares the medicine prize with Dr M.F. Greaves, of Britain's Imperial Cancer Research Fund. The science prize is shared by Professor Ricardo Miledi, of University College, London, and Professor Pierre Chambon, of the CNRS molecular biology laboratory at Strasbourg. S.L.H.

Correction: Not Antarctic

In last week's article by Philippa Lloyd (*Nature* 331, 201; 1988) the headline should, of course, have referred to an Arctic expedition. □



SIXTEEN years after it was abandoned in the Antarctic wilderness, a transport plane used in 1971 as part of the US National Science Foundation's Antarctic Program has been flown back to its base at McMurdo Sound. The extremely dry and cold Antarctic environment has preserved the aeroplane in perfect condition beneath snowdrifts that reached close to the top of its tail fin. It took a team of engineers a month to dig the plane — an LC 130 Hercules — out of the snow. Another five weeks were needed to re-install the engines and flight controls that had been stripped from the plane. It was abandoned after a crash landing. Ten million dollars was spent on the recovery mission, far less than the \$35 million needed to buy a new plane. The Hercules, which arrived at McMurdo base two weeks ago, will now go to Christchurch, New Zealand, for further repair work. When back in action it will bring NSF's Antarctic fleet to a total of seven Hercules. All are equipped with skis so that they can land on glacier ice and snow and they are used to ferry scientists and equipment to and from New Zealand and between Antarctic bases. □

Lords condemn UK space policy

London

THE British government is severely castigated this week for its persistent failure to formulate and announce a positive space policy. The criticisms, contained in a report from the influential House of Lords Select Committee on Science and Technology, will be seen as a vindication of the constantly aired grievances of the wider space community. The report (*United Kingdom Space Policy*, HMSO, London, £7.00) has been compiled from detailed evidence of more than 80 witnesses representing the full range of Britain's interest in space.

On funding, now at £116 million, with 70 per cent going to the European Space Agency (ESA), the report pulls no punches: "The present level of total national spending on space is wrong. It gets the worst of all worlds." It concludes that the optimum level of funding "in present circumstances" should be around £200 million after five years. The proportion of the budget devoted to domestic programmes is too little and must be adjusted "to get better value for money". The target should be for national and ESA spending to be equal.

The report takes the government to task for its delay in responding to a space plan it requested from the British National Space Centre (BNSC). Eventually, last July, the government said that no extra funds were available. The committee says: "A year was too long between the government's receipt of the space plan and its decision of July 1987; and there seems to have been no good reason why it hesitated so long. Its silence was misleading . . .". Furthermore, the decision was arrived at without having apparently consulted industry, something the committee "cannot understand".

The government does gain some support. The committee concurs with the stance taken by trade minister Kenneth Clarke at ESA's November meeting of ministers that Hermes should not be funded by Britain, and that a European manned space programme independent of the United States is misguided. The committee nevertheless advocates that Britain "should keep a foothold in launcher development by supporting Ariane 5, even if man-rated, at the same level of funding as Ariane 4".

Increased private sector spending on space should be encouraged, but the government should give a lead: ". . . it is not reasonable to suggest that, in an area where commercial returns are distant, British industry should step in where the government itself has declined to act.

Simon Hadlington

Lack of telecommunication among satellite companies

Washington

THE US company Pan American Satellite has recently become a squeaky wheel in the international telecommunications world, and it seems that the US government may be inclined to give it some grease. PanAmSat has complained in full-page advertisements in the *Washington Post* and *Financial Times* that it is being excluded from carrying potentially lucrative communications traffic between the United States and Great Britain. Now the US government has agreed that PanAmSat's complaint is justified, and it intends take the matter up on a government-to-government basis.

PanAmSat is seeking to become a so-called separate system, offering transmission of data, television signals and private telephone lines outside the Intelsat system. Intelsat has exclusive rights to international traffic in public telephone switching, but will offer interconnections for separate systems so long as there is no infringement of its exclusive domain.

PanAmSat's chairman, Rene Anselmo, wrote to President Reagan on 29 December last year, pointing out the irony of his company's dilemma. The United States, Anselmo wrote, has lobbied hard for Cable & Wireless, a British company, to be given access to the Japanese telecommunications market as part of a consortium with the US company Pacific Telesis. Cable & Wireless also has landing rights for its signals in the United States.

But it is Mercury, a Cable & Wireless subsidiary, along with British Telecom, that PanAmSat says has effectively excluded it from the British market by refusing to negotiate an operating agreement for landing rights. For its part, a

spokesman for Cable & Wireless says PanAmSat has asked for services relating to transmitting television signals that would violate Cable & Wireless's licence agreement in Britain, so it cannot comply. The spokesman says there have been no negotiations since last summer.

Problems in gaining access to European telecommunications markets are not limited to the United Kingdom. Tim Shea of the Commerce Department Office of Telecommunications says other European countries have been similarly unwilling to allow separate systems to enter their markets. The US government feels that European telecommunications monopolies have been two-faced in their attitude about separate systems. They have refused to deal with companies like PanAmSat, but they have been promoting Eutelsat, a separate system justified on the basis of being a regional system, although it crosses international boundaries.

For PanAmSat there is a special urgency to break the current logjam. Its RCA Astro 3000 spacecraft is due to be launched on Ariane flight V22, now scheduled for April. An agreement with Latin American countries on use of the satellite is unlikely to be lucrative.

Other companies are also anxious to see greater openness in global telecommunications. But the US government is torn. Comsat, the US member of Intelsat and the largest shareholder in the operation, stands to lose if companies such as PanAmSat can skim the cream from heavily trafficked routes. American Telephone & Telegraph is also a heavy user of Intelsat services. So there is significant pressure to leave things as they are.

Joseph Palca

Space University prepares for lift-off

London

AN ambitious new international venture to provide aspiring space scientists with academic tuition across the range of space-related topics is in the final stages of preparation. This summer, the first batch of 100 students from around the world will attend a nine-week multidisciplinary course at the Massachusetts Institute of Technology (MIT) under the auspices of the International Space University (ISU).

The university was launched last April at MIT, with grants of \$170,000 from the National Aeronautics and Space Administration and \$50,000 from MIT. ISU's advisory board comprises an impressive assembly of internationally recognized figures from the space community, including Reimar Lüst, director-general of the

European Space Agency, and Roald Sagdeev, director of the Soviet Union's Institute for Space Research.

Participating nations include China, Japan and several European states. Britain came into the university relatively late in the day, receiving an official invitation to join at the International Astronautic Federation conference last October. The British Foundation for the ISU is trying to raise £30,000 to send six students on the first course. The British Foundation's steering committee chairman, Roy Gibson, former head of Britain's national space programme, sees private sponsorship as an ideal opportunity for space industrialists to demonstrate to the government a willingness to fund space science at a fundamental level. Simon Hadlington

Jet Propulsion Laboratory is considering its options

Pasadena

THE summer of 1989 will be a busy time for the Jet Propulsion Laboratory (JPL) in Pasadena, if everything goes according to plan. Magellan, the Venus radar mapper, will be on its way, Voyager will make the first-ever encounter with Neptune, and Galileo will be only months away from starting on its planetary billiard shot that will eventually take it to Jupiter by way of Venus and Earth. Although those missions will keep planetary scientists busy for some years, JPL must find new projects to carry it through the next decade.

The loss of the space shuttle Challenger brought the US planetary science programme to an abrupt halt. Occurring just days after a fabulously successful encounter with Uranus by Voyager, the accident forced the further postponement of Galileo, a mission JPL has directed from its inception. Galileo was due to arrive at Jupiter later this year, but is now delayed until December 1995.

As NASA's primary centre for planetary science, JPL has been hard hit by Galileo's delays. It is more than 10 years since the United States last launched a mission to the planets. Although increased attention to Mars is high on Reagan's agenda for US space activities, apart from the Mars Observer probe to be launched in 1992, most Mars projects are only in the early development stage.

JPL occupies a unique position among NASA regional centres. Unlike all others, it is operated by an academic institution — the California Institute of Technology — and its 5,300 employees work for Caltech, not the government. This gives JPL scientists flexibility their civil service colleagues lack. But it also means they must ask for more money when seeking grants from NASA, as personnel costs must be added into the budget, instead of being part of the institutional overhead.

Lew Allen, director of JPL, says the country is in danger of losing a valuable resource if JPL is not assigned a new planetary mission. The laboratory has a broad range of disciplines related to manufacturing spacecraft, and had planned to devote its future energies toward the Mariner Mark II spacecraft — designed for sophisticated planetary missions but not as comprehensive a spacecraft as Galileo. But it now seems that the Reagan administration will not give a go-ahead in the 1989 budget to the Comet Rendezvous, Asteroid Flyby (CRAF), the first planned Mariner Mark II mission. Without CRAF or Cassini, its sister mission to Saturn's moon Titan, JPL will have to shift direction in a hurry to find new ways to keep its spacecraft builders busy.

One possibility, according to Allen, is a sample return mission to Mars. A combination of events appears to be conspiring to make Mars the next object of intensive operation. Popular enthusiasm for the planet has always been high, and two reports on NASA's future have recommended trips to Mars as future goals of the agency. There is also the need to come up with something for the International Space Year, set for 1992. NASA will also be under pressure to keep up with the Soviet Union's ambitious Mars plans.

But Moustafa Chahine, chief scientist at JPL, believes it is a mistake to focus too much on Mars. Chahine says the next logical question for planetary scientists to



Lew Allen, JPL's director, making space missions popular.

study is the molecular and atomic evolution of the Solar System. That can be done only with a broad planetary programme aimed at more than one planet.

Despite the uncertain future and launch delays, Chahine says morale at JPL remains high. Enthusiasm for the redesigned Galileo mission is high, and although the new trajectory triples the time needed to reach Jupiter, there will be previously unplanned possibilities for scientific observations of Venus, Earth and two asteroids.

Chahine is also cheered by a new report being prepared for NASA administrator James Fletcher concluding that the quality of scientific research at NASA centres — particularly JPL — is at least the equal of that in universities. But D. James Baker, author of the report, warns that without a strong commitment to planetary science in the future, JPL will be forced into new areas of research — most likely defence-related work — to the detriment of the civilian space programme. **Joseph Palca**

SmithKline cash for Cambridge

Tokyo

A BRITISH attack on the human genome is to be launched by Dr Sydney Brenner with the help of the Medical Research Council (MRC), the Imperial Cancer Research Fund (ICRF) and SmithKline Beckman Corporation, the pharmaceutical company. The company is to give \$2.25 million to the School of Clinical Medicine of the University of Cambridge to which Brenner will move his MRC unit later this year.

For Brenner, the move represents the beginning of the end of a long struggle to find backing for a serious attempt to apply his technique for mapping the genome of the nematode worm *Caenorhabditis* to the much larger human genome.

Until the appointment of Dr Dai Rees as secretary of the MRC last October, the council had seemed reluctant to back Brenner's project and, although he had some other funds available, including his share of last year's Louis Jeantet Foundation prize, they were not enough. The problem has been solved by Brenner, Rees, Sir Walter Bodmer, ICRF's director, and Keith Peters, Regius Professor of Physics at the University of Cambridge.

Of SmithKline Beckman's contribution, \$1 million is to be spent on refurbishing and equipping laboratories at the School of Clinical Medicine and the rest on supporting research over the next five years. Officially, Brenner will advise Peters on the programme of research, with further advice from Dr Tim Rink, who left the department of physiology at Cambridge to become vice-president of research at the company's UK subsidiary, Smith Kline French.

Molecular research into cardiovascular disease, virus infections and autoimmunity will receive priority. The company retains the option to patent and commercialize the outcome of its investment; royalties will accrue to the university.

Exactly how ICRF will contribute to Brenner's mapping efforts has yet to be worked out but is likely to take the form of a group of ICRF-supported scientists working alongside Brenner. There will be close links between the Cambridge scientists and the group of Drs Hans Lehrach and Anna-Marie Frischauf at the ICRF laboratory in London, which has already begun work on human genome mapping.

The result, says Bodmer, should be a powerful and concerted programme of research on the human genome in Europe. Discussions on including other European partners, particularly in France, are under way. Another result, of particular importance to Brenner, is that the project can continue after he has been pensioned off by MRC. **Peter Newmark**

Incidence of leukaemia

SIR—There are several points in the letter by Crouch¹, concerning the paper by Forman *et al.*² on cancer near nuclear installations, that require comment.

First, Forman *et al.* employed cancer mortality data in their paper, rather than incidence data as suggested by Crouch, because “cancer registration data are of variable quality” (p.499).

Second, the observed excess of lymphoid leukaemia deaths in people under 25 years old is confined to what the authors term the “pre-1955 installation” grouping — those establishments other than Sellafield that commenced nuclear operations before 1955. Indeed, Forman *et al.* state (p.501): “For lymphoid leukaemia there is a statistically increasing trend in RR [relative risk] with increasing proximity to an installation in the pre-1955 group (two-sided $P = 0.048$) and a significantly decreasing trend with increasing proximity to an installation for the grouped CEBG [Central Electricity Generating Board] installations (two-sided $P = 0.015$)”.

The authors also note that this high relative risk associated with the pre-1955 installations is due, in large part, to particularly low numbers of observed deaths in the control areas, with which the installation areas are being compared.

Third, Crouch draws attention to radiation doses from the alpha-emitting isotopes of plutonium and americium in areas near Sellafield. The pre-1955 installations consist of the Amersham, Harwell, Springfields, Aldermaston and Capenhurst sites, and Forman *et al.* note (p.500) that “the activities carried out in these installations are heterogeneous”. Springfields and Capenhurst are uranium-processing factories which do not handle transuranium radionuclides³; Amersham does not discharge these elements⁴; and the nature of the processes at Aldermaston and Harwell are such that these two establishments release only small quantities of plutonium⁵. It is very unlikely that the observed excess of young lymphoid leukaemia deaths in this installation grouping could be due to emissions of these alpha-emitters.

In addition, Darby and Doll⁶ have found “no convincing evidence” (p.606) that the alpha-emitters released into the atmosphere by above-ground weapons tests have affected the leukaemia rates for this age group in either Britain or Scandinavia.

Considerable caution needs to be exercised in the interpretation of the patterns of leukaemia data near nuclear sites⁷, particularly because mortality from other cancers is lower in the installation areas than in the control areas⁸. Areas around fossil-fuelled power stations (and

suitable control areas) might provide a useful data set with which the results for nuclear installations could be compared⁸.

The impression that the associations found by Forman *et al.* represent causation could prove misleading. I doubt whether Crouch would see one of the benefits of CEBG nuclear reactors as protecting the local populations of young people against lymphoid leukaemia, even though this is what the data for this group of installations would appear superficially to suggest.

RICHARD WAKEFORD

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SIR—Although television current affairs programmes are not normally the most reliable source of information on controversial topics, I should like to draw the attention of David Crouch (*Nature* **330**, 319; 1987) to a recent World in Action programme on child leukaemia.

Crouch asserts that child leukaemia “could indeed have been caused by radiation, and that we know of no other likely cause”. Quoting an apparently reliable researcher in Newcastle, this television programme identified a ‘super cluster’ of excess child leukaemia in Gateshead, which is nowhere near a source of radiation.

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Whiggishness

SIR—I would like to add a vote for the ‘pro-Butterfield’ side of the debate on scientific whiggishness (*Nature* **329**, 213; 1987).

Few would disagree that there are, for example, considerable differences between our scientific view of the world and that prevalent in ancient Egypt. These differences are unlikely to have come about overnight, old perspectives being discarded at the breakfast table. Our personal experience of a continuity of

perspective suggests that such changes are of a more gradual evolution. Yet, like growing old, the subtlety of the change can blind us to its occurrence. Our modern perspective is not that of the nineteenth century, less that of the eighteenth, and it is uncertain how reliably one perspective can interpret another.

There is disagreement, even now, in matters of politics and religion. But a world view is a more profound level of perception than is membership in a political party. How can one truly understand a differing existential perspective in a more than rhetorical sense? One viewpoint is felt to be real and one necessarily lacks conviction — and without conviction it becomes no more than one pole of an argument.

Our science and our view of the world are closely interrelated. Even allowing the postulated Zen-like state where scientific history is chronicled with an ‘empty rather than a full mind’, the history of science is fraught with perils.

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Soviet psychiatry

SIR—I would like to correct a number of points in the article by Joseph Palca and Vera Rich on Soviet psychiatry (*Nature* **330**, 684; 1987).

Professor Aleksandr Snezhnevskii, who died recently, was the former head of the Institute of Psychiatry in Moscow, not of the Serbskii Institute of Forensic Psychiatry. He wrote widely on schizophrenia, much of the material being available in the West, and he never described all mental illness as a form of schizophrenia. His *Handbook of Psychiatry* (Meditsina, Moscow, 1983) covers the same range of topics as any Western psychiatric textbook. Incidentally, the chapter on schizophrenia in this book summarizes much of his previous work, and includes the criteria for diagnosing schizophrenia. He has never described political dissent as a form of “creeping schizophrenia”. This notion has been perpetuated through ignorance or distortion of his work. There seems to be a basic failure to distinguish between the form and content of delusional thinking, a mistake which is common to students at the beginning of their psychiatric training.

Dialogue between Soviet and Western psychiatrists is going to be difficult while we continue seriously to misrepresent the views of a leading and highly respected Soviet psychiatrist.

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Japan as a high-energy power

Japan's new electron-positron collider seems to have quickly won itself a reputation for excellence, showing that the familiar excellence of Japanese engineering can bring academic benefits.

Tsukuba

ATTENTIVE readers of the international physics journals will have noted in the past few weeks a spate of papers from the new electron-positron collider TRISTAN now operating here. On the leapfrogging principle that new machines usually produce their most interesting data in that brief interval before they are overtaken in performance by another, that is not in itself surprising. But TRISTAN's demonstration of how to hit the ground running seems to have bettered what has been done elsewhere by a substantial margin. What can be the explanation?

Although there are many in Japan who regard TRISTAN as a proof that the country is interested in basic science, not merely in profit-making technology, the machine is also a talisman of the Japanese scientific community's willingness to throw resources into the international collaborative melting-pot.

TRISTAN has also slipped into being an object of national pride. Dr Tishi Ozaki, director of the project, explains that his colleagues worked on the commissioning of the machine in the spirit of "we must succeed". A practical side-benefit is that the enthusiasm appears to have spread to the industrial companies contracted for construction and the supply of equipment; Professor Tetsuji Nishikawa, director-general of the National High-Energy Physics Laboratory, explains that, in Japan, if the design team knows what it wants to have made, construction companies will struggle to be given the chance to do the work. An American fellow visitor marvels at this state of affairs, saying that contractors in the United States regard the academic community as a source of profitless fiddling work.

The first machine in Japan on this scale, may, of course, be an exception. But that seems improbable. One of the familiar, if elsewhere unexpected, features of Japanese life is that academics who know what they want are respected by their colleagues in industry even if they are less well paid. Tsukuba's TRISTAN is a monument to the success of this formula. The academics' reward is not so much money as the international respect that will be their due. TRISTAN's staff is already dreaming of the detectors it will be asked to build for the US Superconducting Supercollider, the site of which has not yet

been chosen (see page 289 of this issue). For the time being at least, the people here are saying that there is no question of their building a successor to this machine, but that international collaboration will become the only way forward in high-energy physics.

Meanwhile, Japan's approach to high-energy physics seems delightfully empirical. The great names of the past — Yukawa and Tomanaga — are those of theoreticians. Nishikawa is quick to say. Ozaki is not sure how many experimenters there may now be, but thinks they may amount to 500, some 300 of them students. "There must be three or four times as many theorists", he says. But he is also respectful of the machine-building tradition in Japan, pointing out that there was a substantial cyclotron at the University of Tokyo before the Second World War, that the war itself was a "great interruption" and that the builders of the machines nevertheless got to work again soon afterwards.

Ozaki does not even accept the proposition that the Japanese community may need more help than most with the design of modern detectors. While many of the Tsukuba team have done time at US laboratories — Ozaki himself was for many years at Brookhaven — things are changing so fast, and so much now depends on the software, not the hardware, that he and his colleagues are confident of their self-sufficiency.

Ozaki is convinced that he has the best-instrumented electron-storage ring in the world — there are a dozen computers behind the wall of the control room, and another twenty distributed around the ring. He insists that designers of detectors for the ring should give precedence to the design of their software.

The government, meanwhile, is proud of its deliberate investment of funds over the past decade in the most esoteric kind of basic research, so much so that it has sanctioned a 28 per cent increase of the operating budget for 1988 that will, among other things, allow an increase of operating time by a half to 1,200 hours (see page 289 of this issue). For the politicians, what matters about TRISTAN is that it is a demonstration that Japan is able successfully to invest in basic science. It would, of course, have been more human to have made the investment and to have

made a hash of it, but that is not the Japanese tradition.

What are the results? TRISTAN (roughly a kilometre in diameter) has four equidistant experimental halls around the circumference, one of which is occupied by a simple device of laminated plastic designed to look for magnetic monopoles, whose production cross-section must be very small at 27.5 GeV. That, Ozaki says, is a long-term investment, even a gamble. There is nothing much to report so far. Otherwise, there are three general purpose detectors of the particles possibly produced in electron-positron collisions, each operated by a different university collaboration. One of them, interestingly, involves not merely experimentalists from the United States, but also people from mainland China and from Korea.

The outcome, so far, is that there is no sign of the top quark at a centre of mass energy of 55 GeV, and no signs of exotic electron-like leptons other than muons and taus below the same or a slightly smaller energy threshold. The difference of the collision energy at Tsukuba and Hamburg in West Germany (25 per cent) is not so great as to keep theoreticians awake at night, but it must sharpen in many minds the still unanswered question "Why is the top quark so much heavier than the others?" Meanwhile, largely because of the quality of the software built into the detectors, it seems likely that TRISTAN will have a field-day in unravelling the hadronic products of quark disintegration, which show up nicely on computer reconstructions as opposing streams of particles.

But what will happen after the Stanford Linear Collider (which is already late) eventually comes on line, with LEP at CERN a few months later, in the middle of next year? Nobody at Tsukuba is taking any bets. Ozaki believes that the lifetime of his machine is "between five and twenty years".

But it is most probable Japanese high-energy physicists, having cut their teeth on this successful project, will somehow manage to stay in the game. They have fallen into the traditional trap for the Japanese of making things work. They have also built a high-energy physics laboratory that differs from all others in its dazzling cleanliness, which is something to be pleased about in itself.

John Maddox

Sensory transduction

A taste of things to come

John Teeter and Geoffrey H. Gold

TASTE qualities are commonly divided into four categories: sweet, sour, salty and bitter. Yet these categories include such chemically diverse substances as sugars, acids, salts and alkaloids. How do taste-receptor cells (Fig. 1) detect and distinguish between such a variety of substances? Several mechanisms will probably be needed fully to explain gustatory (taste) transduction; Avenet *et al.*¹ and Tonosaki and Funakoshi² on pages 351 and 354 of this issue, respectively, report that one of these mechanisms may use cyclic nucleotides as intracellular messengers.

Like all sensory receptors, gustatory receptor cells transduce sensory stimuli into changes in membrane potential. Elucidating the mechanism of gustatory transduction, however, has turned out to be particularly difficult. One reason for this is that only the small apical surface of the cell interacts with chemical stimuli (Fig. 2b). Another reason is that several different transduction mechanisms may be used by gustatory receptor cells. Taste responses to sodium and lithium salts, for

example, are blocked by the sodium-channel blocker amiloride³. This result suggests that salts depolarize the receptor cells, in part by the entry of stimulus cations through passive (ungated) channels in the apical membrane (Fig. 2a). In contrast, other evidence indicates that acids, which taste sour, depolarize receptor cells by a hydrogen-ion block of potassium channels^{4,5}. Some other substances may depolarize the receptor cell by an allosteric effect on ion channels, as L-arginine specifically gates ion channels in reconstituted gustatory membranes⁶.

In contrast to the evidence mentioned above for the direct effects of taste stimuli on ion channels, intracellular second messengers may also contribute to gustatory transduction. Bitter stimuli, for example, induce a rise in intracellular calcium concentration in rat taste cells, and the source of the released calcium is intracellular⁷. This observation implicates the inositol phospholipid pathway, as inositol trisphosphate, one of the products of inositol phospholipid hydrolysis,

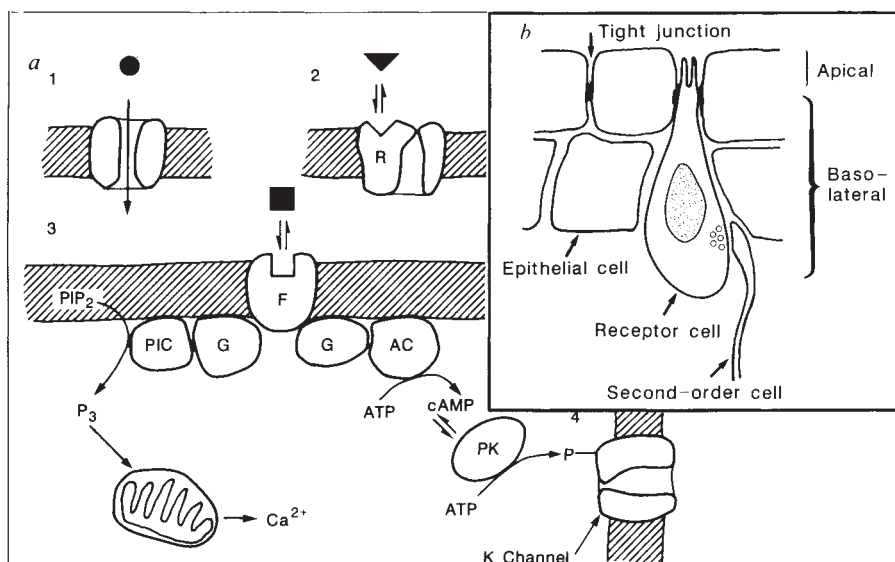


Fig. 2 *a*, Possible mechanisms of stimulus-induced depolarization of receptor cells. ●, ▼, ■ indicate stimulus molecules. 1, Salt stimuli may depolarize the cell by entry through passive (ungated) channels in the apical membrane. Acid (sour) stimuli may inactivate voltage-dependent potassium channels in the apical membrane (not illustrated). 2, Amino acids binding to membrane receptors (R) may induce channel opening allosterically. 3, Other stimuli, such as sweet and bitter, may bind to membrane receptors, activating G-protein (G)-mediated production of intracellular messengers such as cyclic nucleotides (cyclic AMP, cAMP) and inositol trisphosphate (IP₃). Cyclic AMP is synthesized by the enzyme adenylate cyclase (AC). Inositol trisphosphate is produced by the hydrolysis of the membrane phospholipid phosphatidylinositol-bisphosphate (PIP₂) by the enzyme phosphoinositidase C (PIC). 4, Cyclic AMP may initiate an electrical response by causing a block of potassium channels in the baso-lateral membrane via protein kinase (PK)-mediated phosphorylation. *b*, Mammalian taste bud. Taste stimuli interact with the exposed apical surface of the receptor cells to initiate membrane depolarization and synaptic transmission to second-order neurons. Taste stimuli are restricted to interacting with the apical surface of the cells by a layer of tight junctions between the receptor cells and the surrounding epithelial cells.

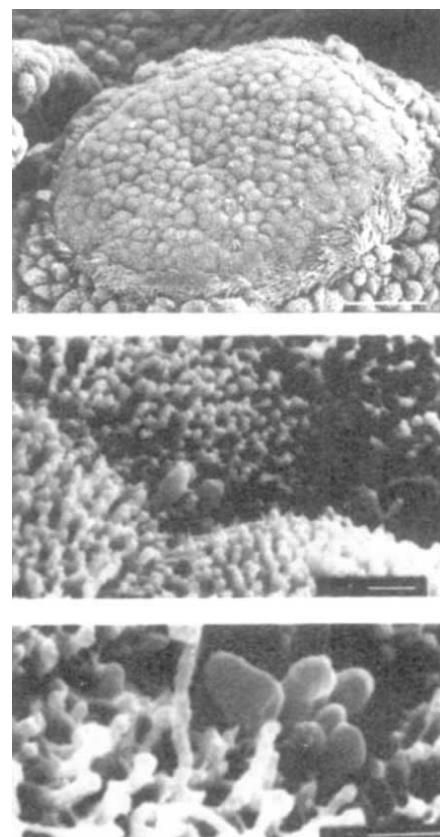


Fig. 1 Top, taste bud from the tongue of a frog, viewed from the mucosal space. Scale bar, 30 μ m. Middle and below, apical projections of taste-receptor cells. Scale bars, 1 μ m. Scanning electron micrographs by Professor Pedro Mestres.

releases calcium from intracellular stores in most cells. Consistent with this possibility, stimulus- and GTP-dependent activation of inositol trisphosphate formation has been seen in catfish gustatory membrane preparations⁸. Cyclic nucleotides may also be intracellular messengers in gustatory transduction, as stimulus- and GTP-dependent cyclic AMP synthesis has been reported in both rat⁹ (with sugars) and catfish¹⁰ (with amino acids). In this respect, gustatory receptors resemble vertebrate photoreceptors, which use cyclic GMP to mediate sensory transduction¹¹. An interesting parallel between visual, olfactory and gustatory receptors is that stimulus activation of both the inositol phospholipid and cyclic nucleotide pathways has been observed in all three receptor types. Yet in photoreceptors, the best understood sensory transducers, inositol phospholipids seem not to be involved in the transduction process. Because it is not yet possible to separate different taste-cell subclasses for biochemical studies, it is not known whether the inositol phospholipid and cyclic nucleotide pathways coexist in the same cells.

Electrophysiological studies on gustatory receptors are now helping to clarify the biochemical results. The particular advantage in making electrophysiological

measurements is that the output of the transduction process can be measured. One can therefore differentiate between a host of cellular processes, only some of which trigger the electrical response to sensory stimulation. In their report in this issue², Tonosaki and Funakoshi describe experiments in which they injected putative intracellular messengers into mouse taste cells. They find that cyclic GMP, and to a lesser extent cyclic AMP, decrease potassium conductance, leading to membrane depolarization. They also show that sucrose depolarizes the same cells via a decrease in potassium conductance. Thus, the data support the hypothesis that detection of sugars is mediated by sucrose stimulation of adenylate (and/or guanylate?) cyclase. The calcium chelator EGTA also depolarizes the cells, but this may result from the well-known inhibition of adenylate cyclase by calcium, rather than indicating that calcium itself is the intracellular messenger. Similar results have recently been reported by Okada *et al.* (ref. 12).

Avenet *et al.* in their paper in this issue¹ describe the use of the patch-clamp technique to investigate the effects of cyclic AMP on frog taste-cell conductances. Like Tonosaki and Funakoshi, they find that cyclic AMP decreases potassium conductance. Avenet *et al.* did not test the effects of cyclic GMP, so we do not know if the preferential effect of cyclic GMP observed by Tonosaki and Funakoshi is unique to their preparation. Avenet *et al.* also investigated how cyclic AMP decreases potassium conductance, and find that it acts via protein phosphorylation rather than via direct gating of the ion channels. This is perhaps surprising, as cyclic nucleotides control conductances in both photoreceptor and olfactory receptor membranes by a direct gating action¹³. However, this mechanistic difference is correlated with the neural origin of visual and olfactory receptors as compared with the epithelial origin of gustatory receptors.

During the past few years, there has been rapid progress in elucidating the

mechanisms of sensory transduction. Visual transduction in vertebrates, for example, is now known to be mediated by a light-stimulated enzymatic cascade which reduces intracellular cyclic GMP concentration; cyclic GMP-gated channels then translate the decrease in cyclic GMP concentration into a decrease in conductance¹¹. Recent evidence suggests that olfactory transduction is mediated by

a similar mechanism¹³. Perhaps, therefore, the work summarized here on gustatory receptors is only a taste of things to come. □

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Meteorites

Evolution of ureilites

Martin Prinz

UREILITES, one of the most enigmatic of meteorite groups, are represented by over 30 specimens, two-thirds of which were found in Antarctica. They consist mainly of olivine, pigeonite and carbon (including diamond and/or graphite) and have engendered controversy with each new study. What is the origin of the diamonds? Why are there large amounts of noble gases concentrated in the carbon-rich veins? Did the carbon coexist with the silicates during crystallization or was it injected later? Are the silicates of cumulate or residua origin, and are the ureilites achondritic, chondritic or both? There is general agreement that ureilites are related to some carbonaceous chondritic precursor, but a new controversy has now arisen. On page 321 of this issue¹, Grady and Pillinger show that the newly recognized group of polymict ureilites is highly enriched in ¹⁵N, in contrast to the main-group ureilites. Such enrichment is found in only a few unusual meteorites and their relationship with other ureilites is not at all clear.

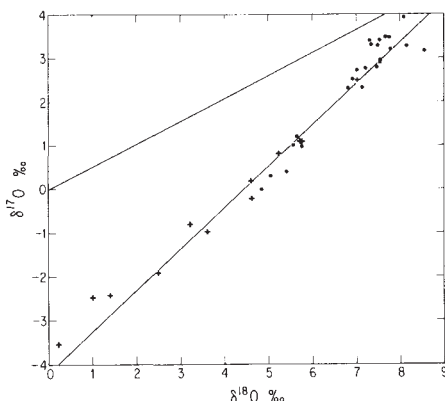
Three polymict (or multi-component) ureilites have been recognized and, although they contain many lithic fragments, they consist mainly of ureilitic

material similar to that already known. A deeper understanding of these components would help to elucidate the origin of ureilites, particularly if they are shown to be related. This does seem to be the case, as demonstrated by the new oxygen isotope studies² of Clayton *et al.*. One question emerging is, if the ¹⁵N enrichment is found only in the polymict ureilites, does it represent a foreign projectile component unrelated to the ureilites? This is what Grady and Pillinger hypothesize¹.

Most meteorites are chondritic and are primitive samples reflecting processes in the early Solar System, whereas others are achondritic and formed as molten rocks in planets developed from the chondrites. Ureilites have previously been considered to be achondrites, but the new oxygen isotope data make this uncertain. Clayton *et al.* plotted² a 'three-isotope' graph — the ¹⁷O/¹⁶O ratio against the ¹⁸O/¹⁶O ratio — for the measured contents of various ureilites, and found that the data are scattered about a straight line of slope one (see figure). A slope of a half would indicate mass-dependent mixing of the isotopes by igneous differentiation from a common source. Instead the unit-slope line common to calcium–aluminium-rich inclusions (CAIs) and dark inclusions in carbonaceous chondritic (CV3) meteorites implies that ureilites were formed by local melting of an isotopically heterogeneous precursor somehow related to the dark-inclusion material. Therefore the ureilites cannot be igneous rocks formed from a common magma.

Clayton *et al.* also measured five different portions of the Nilpena polymict ureilite, including a ¹⁵N-rich carbonaceous matrix clast, and all fall on or near the same trend line. This indicates that the lithic components in polymict ureilites are indeed related to the ureilites. Thus, the ¹⁵N enrichment of polymict ureilites, but not of ureilites, presents a dilemma.

The data of Clayton *et al.* also present severe problems for two other recent ureilite studies. One, by Goodrich *et al.*³, develops a complex scheme of multistage igneous activity on a large parent body.



Three-isotope graph, showing the content of ¹⁷O against that of ¹⁸O for several ureilites (dots). They fall around a line of slope 1 common to dark inclusions in C3 chondrites (crosses, the Allende mixing line). The slope-1/2 line above is that found for rocks from the Earth. (Courtesy of R. N. Clayton.)

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The other, by Janssens *et al.*⁴, uses trace elements to decipher the planetary history of ureilites by means of a cumulate model which they have modified. Both papers were published just as the new oxygen isotope data were becoming available.

Goodrich *et al.* argue that ureilites are cumulates with an igneous history as complex as that of the Moon. The ureilitic magmas had superchondritic Ca/Al ratios and negative europium anomalies requiring a plagioclase-depleted source. An earlier melting event, according to Goodrich *et al.*, formed a differentiated crust, and later remelting of mafic cumulates resulted in ureilitic parent magmas from which cumulates formed. They find that the planet did not have a core and had a radius of at least 235 km. They even speculate, although cautiously, that the parent planet could be Venus. There is, they say, evidence for varying degrees of reduction of iron oxide in the parent magmas for each ureilite and, because graphite is considered to be the reducing agent, each ureilite equilibrated at different pressures. This complex model is difficult enough to establish for any postulated planet, but has the problem created by the oxygen isotope data showing that these samples cannot be linked by any igneous differentiation model.

The valuable trace-element data⁴ presented by Janssens *et al.* are also important for determining ureilite genesis. These authors find evidence for two metal components, one in the carbonaceous veins and the other in the ultramafic silicates. They also find evidence for melting and incomplete segregation of metal, iron sulphide and carbon, and suggest that the noble gases expelled by the cumulate process may have been retrapped during magmatism if the system was closed. Clearly, this planetary model also faces the challenge of the Clayton *et al.* oxygen isotope data.

Even the origin of the diamonds in ureilites, long considered to be of shock origin, is being challenged. Fukunaga *et al.*⁵ synthesized diamonds by chemical vapour deposition and suggested that diamonds in ureilites formed directly from the solar nebula rather than by shock. Further studies of the genesis of diamonds by vapour deposition in carbonaceous chondritic meteorites are now under way and should provide insight into planetary origins. □

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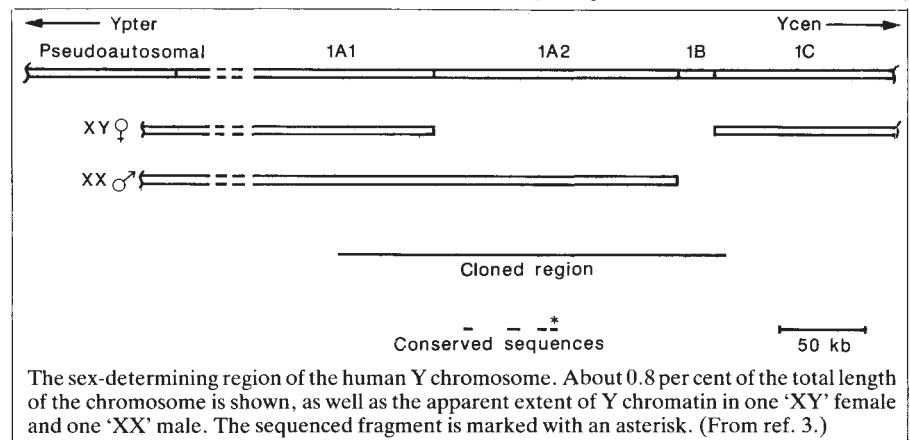
Developmental genetics

Everything you always wanted to know about sex . . .

Jonathan Hodgkin

SINCE 1959 it has been known that sex in man is determined by the presence or absence of a Y chromosome^{1,2}. More recently it has become clear that only a small region of the Y, perhaps a single gene, is responsible for initiating the sequence of events that lead to testis formation and hence to male development. In the absence of this gene, called TDF (for testis-determining factor), female development ensues. Page, de la Chapelle and collaborators³ have now cloned a 230-kilobase (kb) segment of the human Y chromosome, which must

specificity in other mammalian species, and four such sequences were found by probing male and female DNA from various mammals, using 'Noah's Ark' blots. Moreover, a mouse Y sequence related to one of these was detected in DNA from *Sxr* (sex-reversed) XX male mice⁵, strongly supporting the belief that it comprises part of TDF. The corresponding human restriction fragment (1.3 kb) has been sequenced. Page *et al.* do not report the nucleotide sequence, but find that in one reading frame there is an uninterrupted coding sequence of 404 amino acids.



include some or all of the sex-determining region; they have identified evolutionarily conserved sequences within this region, and they have sequenced one of these, which appears to encode part of a nucleic acid-binding protein. It is very likely, though not yet proved, that this is TDF.

The sex-determining region of the Y chromosome was defined by analysing natural human variants: individuals with two X chromosomes who develop as males; and individuals with an XY constitution who develop as females. About one in 20,000 men is an XX male; they are sterile, and consequently have been identified by presentation at fertility clinics. Most of these XX males are now known to carry a small part of the Y chromosome⁴. Conversely, some XY females carry small deletions of the Y. The most informative of all the individuals so far examined are one XY female who appears to carry all but 140 kb of the Y, and one XX male who carries less than 350 kb of the Y. Together, these cases show that 'interval 1A' (see figure) is both necessary and sufficient to confer maleness.

Functionally important sequences within interval 1A would be expected to show evolutionary conservation and Y

composed of 13 repeats of 28–32 residues. The structure is unmistakably that of a 'finger protein': within each repeat there are a pair of cysteines and a pair of histidines, which together with a coordinated zinc atom are expected to create a nucleic acid-binding domain, or 'zinc finger'.

At this point, one cannot predict whether the putative TDF protein exerts its effects by binding to DNA or to RNA (the archetypal finger protein, transcription factor TFIIIA, does both⁶). It is also entirely unknown what the target sequence or sequences might be. Here the work arrives at a difficulty all too familiar to those who work on oncogenes or homeotic genes: a regulatory protein (whether it be a protein kinase or a DNA-binding protein) can be studied *ad nauseam* with little idea of what cellular functions are being regulated *in vivo*. Nevertheless, the nature of the predicted protein makes it likely that TDF exerts its initial effects at the level of a single cell, which is a somewhat unexpected finding.

Formal proof that this gene is indeed TDF must await further experiments. The most compelling of these would be the construction of transgenic mice carrying a complete TDF gene, which should behave

like *Sxr* mice. A bovine complementary DNA which hybridizes to three of the four conserved regions has now been obtained, so such an experiment may not be far off.

Two other aspects of the work are particularly interesting. First, there seems to be a similar gene on the X chromosome, both in man and in all the other mammals examined. Page *et al.* argue that this may be functionally related to TDF and that the high degree of conservation makes it unlikely that this is a pseudogene. They speculate that the X gene could act antagonistically to TDF or, alternatively, that the two gene products could act in concert as a heterodimer in males, whereas the X gene product alone would act in females. They also discuss a more radical possibility, which would unite the processes of X-chromosome inactivation and sex determination⁸. If X and Y genes perform identical functions, and are expressed at the same level, then X-chromosome inactivation would leave one active gene in XX and two in XY. This dosage difference, rather than the presence or absence of the Y gene, could act to direct to subsequent events. Dosage mechanisms of this type are involved in sex determination in some invertebrates, such as fruitflies⁹ and nematode worms¹⁰, but there is no reason to expect them to be universal.

Second, the putative TDF probe detects a related sequence in chicken DNA, although there are identical hybridization patterns for both roosters and hens. In birds, the female is the heterogametic sex (ZW), whereas males are ZZ, so the bird gene, if it is also male-determining, must be regulated in a different way from mammalian TDF. Furthermore, if the gene is present in birds, then it is probably also present in reptiles, which exhibit further variations in sex-determination mechanisms. In fact, ancestral tetrapod sex-determination may have been environmental rather than chromosomal: many turtles and crocodiles exhibit temperature-sensitive sex determination¹¹. Perhaps the original TDF protein was temperature-sensitive in function, and came under different kinds of regulation with the evolution of homeothermy in mammals and birds. In any event, the isolation of this key gene should unlock the door to the understanding (and exploitation) of primary sex determination in all mammals, and possibly in other vertebrates. □

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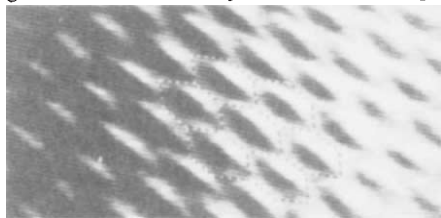
Scanning tunnelling microscopes

Atomic-scale engineering

J.B. Pethica

ATOMIC-RESOLUTION images of surfaces are now regularly obtained using the recently developed scanning tunnelling microscope (STM). Efforts have also been made to alter the structure of the surfaces using the STM as a high-resolution probe. It has thus become one of the principal *gedanken* tools for nanotechnology — the proposed direct manipulation of matter, especially biological, on the atomic scale. On page 324 of this issue¹, J.S. Foster and colleagues demonstrate the pinning of small organic molecules to a graphite surface, and the partial ability to dissect these molecules. These appear to be the smallest scale structures to be made and imaged by the STM to date.

Writing of near atomic scale structures was demonstrated² last year, and I discussed it in a News and Views article³ at the time. Very small groups of germanium atoms were placed on an otherwise flat germanium surface by means of a voltage



STM image of graphite surface showing hexagonal unit cell (from ref. 10).

pulse; they were subsequently 'read' using the STM in its imaging mode. In the new work¹ of Foster *et al.*, graphite is the flat substrate onto which features are written. The graphite is covered with a small drop of di(2-ethylhexyl) phthalate and the STM tip brought to the graphite through this organic liquid. A voltage pulse is then applied, and immediately thereafter a feature about 1 nm across appears on the STM image of the graphite surface. The feature is attributed to an organic molecule becoming pinned on the graphite surface. The authors suggest that the molecule becomes pinned because the voltage pulse excites the molecules, giving charge transfer or bond breaking. This could also alter the unoccupied electronic levels of the graphite, allowing the molecule to be imaged. Under some circumstances it was possible to create structures only 0.4 nm across, the smallest deliberate structure yet made.

Having created a feature on the graphite, it can be altered by applying a further voltage pulse when the tip is again over the feature. This results in enlargement, partial erasure or complete removal of the feature, at present unpredictably. The partial erasure reported by Foster *et al.*

implies that molecules may have pieces deliberately removed, and in principle be atomically 'edited', thereby demonstrating one of the ideals of nanotechnology.

The work has a number of intriguing features. First, the type of molecule does not appear to be important, successful writing being obtained with a variety of organic species. This implies that the binding mechanism may be generic to organic materials, such as the hydrogen and oxygen binding to graphite that Foster *et al.* suggest. Second, writing and altering the structures requires about 3.5 V, similar to the C-C bond energy. This is also the voltage, around the value of the work function, near which field emission or evaporation, or dielectric breakdown may occur across the narrow tunnel gap. The polarity independence also suggests a field or impact effect rather than a bond specific mechanism. Last, it should be noted that the work is exclusively on graphite. This gives very clear-cut and elegant images of the graphite lattice, which are known, however, to have some rather unusual characteristics — excessively high image corrugation heights for the known charge density, and freedom from single-atom scale defects. Some models for these anomalous effects (applicable also to other layered materials) have been proposed⁴⁻⁶ which may involve mechanical contact of the tip and graphite surface^{6,7}. The tunnelling occurs over an area rather larger than a single atom (see figure). The features seen by writing could thus be caused by voltage-pulse induced alteration of the graphite itself; small features the size of the contact area would be produced and altered.

Progress is being made in a number of applications of surface alteration using the STM. Structures have been written on the 10-nm scale or greater in amorphous metals⁸ and in standard electron-beam lithography resists⁹. The work² on germanium is closer to the atomic scale. The work of Foster *et al.*¹ represents a significant attempt at the much more important and difficult problem of the direct manipulation of the structure of biological materials. □

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Natural radiation

How to live with radon

Michael O'Riordan

'As safe as houses' is not a sound simile. Mortality statistics for accidents in homes show that one death in a hundred is from fumes, falls or fires. If potential deaths from indoor exposure to radon are included, home seems even less a haven. Participants at a recent meeting* agreed that radon is the most serious cause of human irradiation, attempted to quantify the consequences for health and explored the developing controls on domestic exposure. It is clear that an important advance is occurring in radiation protection — the extension of dose limitation from artificial to natural sources.

The risks from radon are as real as those from any other radiation, and doses received by the public can be much higher than from artificial sources; excessive exposures are, moreover, readily avoidable. These facts should be reflected in a rational approach to radiation protection, but public perception determines otherwise, and effort is perforce diverted to less serious sources. It was encouraging to learn at the meeting that the imbalance is being corrected.

Human exposure to terrestrial gamma rays, cosmic rays and natural radionuclides in diet is appreciable but not too variable. The annual dose from these sources of radiation, which has been called basic background, is on average about 1 mSv (millisievert), this quantity being the effective dose equivalent. It is important to establish the magnitude of this dose, as it is a convincing criterion of the acceptability of the annual limit of 1 mSv set by the International Commission on Radiological Protection (ICRP) for prolonged exposure to artificial sources of radiation.

Variability

The chief characteristic of indoor radon levels, however, is their variability. On average, the concentration in temperate latitudes is about 15 Bq m⁻³ (becquerels per cubic metre of air), the equilibrium equivalent concentration of radon-222. The value of this quantity can range from an order of magnitude below to three orders of magnitude above for otherwise ordinary houses.

Dosimetric models for the human lung lead to a conversion coefficient of 10 Bq m⁻³ = 1 mSv yr⁻¹ between the annual average concentration and the annual effective dose equivalent (Anthony James, National Radiological Protection Board, Chilton), and there are corroboratory re-

sults from studies of the rat lung (Naomi Harley, New York University School of Medicine). Annual doses, therefore, run from 1 mSv or less through an average of 1.5 mSv to 1,000 mSv or more. The gravity of this circumstance has not escaped notice, and enlightened authorities are following the recommendations of the ICRP by intervening to remedy and prevent the highest indoor exposures.

The high indoor levels are caused by the forced flow of radon-laden soil gas into buildings. The entire process is described by a 'four-p' mnemonic: production of radon from the trace quantities of radium in the ground; permeability of the ground to air; perforations or other gaps in floors; pressure differential between houses and the atmosphere, which is usually negative (Anthony Nero, University of California, Berkeley).

The four immediate decay products, or

Radon reference data from the ICRP			
Radon parameter	EEC (Bq m ⁻³)	Lifetime risk	
		Relative	Absolute
		(per cent)	
Average value	15	0.1	0.25
Upper bound	100	0.5	1.3
Action level	200	1.0	2.5

Radon parameters are the prevailing, prevention and remedial levels for houses. EEC implies lifelong exposure at equilibrium equivalent concentration. The absolute value of lifetime risk is for a population with 2.5 per cent prevailing risk of lung cancer.

daughters, of radon-222 are radioactive isotopes of solid elements with short half-lives, two of which transform by emitting alpha particles. The daughters create a radioactive aerosol with small particles in room air. When inhaled, some daughters are deposited and retained in the respiratory tract, where the alpha particles irradiate epithelial cells, particularly in the bronchial region; the potential effect is the induction of lung cancer. Radon gas itself delivers only a very small dose.

To date, there is no conclusive evidence of excess lung cancers among households exposed to high levels of radon daughters, but more epidemiological investigations are being conducted in Sweden, Norway, Britain and the United States, and others are being planned. Case-control studies preponderate, each requiring the establishment of past exposure to radon and allowance for cigarette smoking. There is, however, determinative evidence from epidemiological studies of miners who received large doses underground, supported by the results of inhalation

studies on animals (Frederick Cross, Pacific Northwest Laboratory, Richland). A relative risk model with a multiplicative influence of smoking has been used by the ICRP, after appropriate adjustment, to translate the lifetime risk of lung cancer from mining to domestic exposure (Wolfgang Jacobi, GSF Institut für Strahlenschutz, Munich). In the table, the model is applied at the average, design and action levels for indoor radon recommended by the ICRP. Lifelong exposure at 200 Bq m⁻³, for example, implies a doubling of the prevailing risk, which is 2.5 per cent for the reference population, but which varies from country to country.

Programmes

The Committee on the Biological Effects of Ionizing Radiation (William Ellett, National Research Council, Washington DC) also used a multiplicative model of smoking and radon exposure in a modified relative risk analysis of the data for miners, but assumed that the results are best applied without adjustment to domestic exposure. The outcome is a greater estimated risk than that of the ICRP; the difference is within the uncertainties of the models.

Programmes to determine and limit indoor exposure to radon are being pursued by Nordic, US and British authorities, and the Commission of the European Communities is teetering on the edge of a decision (James McLaughlin, University College Dublin). None of the limitation criteria coincides with the ICRP recommendations for new and old houses, but they are generally congruent. Sweden is the most advanced country in Europe: measurements have been made in 60,000 or so houses, about 2,000 have been remedied, and preventive measures are effective in almost 95 per cent of cases (Gun Astri Swedjemark, National Institute of Radiation Protection, Stockholm). But the most dramatic advances have occurred in the United States, where a partnership is being forged between the public and private sectors to tackle radon (Richard Guimond, Environmental Protection Agency, Washington DC). Federal expenditure on radon reached \$10 million in 1987 and is likely to increase: the main objectives of the programme are to discover the scale of the problem, develop both remedial and preventive measures, and disseminate helpful information. Fundamental research on radon effects is supported, expenditure by states is also substantial, more than 250,000 householders have tested their homes, and a few thousand have remedied high levels. □

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*Fourth international symposium on the Natural Radiation Environment, LNETI, Sacavem, Lisbon, 7-11 December 1987.

Evolutionary ecology

Benevolent sisterhood

Jon Seger and Eric L. Charnov

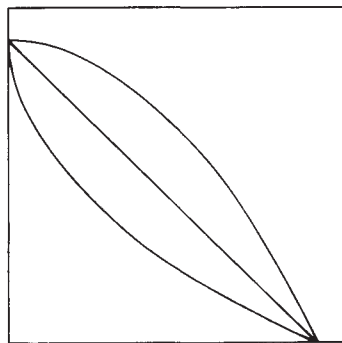
A PRINCIPLE of diminishing returns was first applied to sex-ratio evolution by Hamilton¹, who pointed out that if females usually mate with their brothers, parents should produce the minimum number of sons required to guarantee insemination of their daughters. The reason is that brothers are genetically equivalent from a parent's point of view, so that nothing is gained by having them compete among themselves for matings. This situation is referred to as local mate competition (LMC). It is common in various groups of insects that delight theoreticians by producing female-biased sex ratios remarkably like those predicted on the basis of fairly simple models^{2,3}. (For a recent example published in *Nature*⁴, see Herre's comparative study of facultative sex-ratio variation in fig wasps.) On page 346 of this issue⁵, Schwarz describes what may turn out to be a case of simultaneously diminishing and increasing returns.

LMC is a particular case of what can be called 'differentially nonlinear returns' on investment. Several different ecological causes of nonlinearity have been identified, each with different consequences for the sex ratio, depending on the way in which male and female offspring are affected². In each case the expected fitness of offspring belonging to one sex depends more strongly on the number of individuals of that sex in a brood, than does the fitness of members of the other sex. Under such conditions the equilibrium ratio of investment over the population as a whole may be far from 1 : 1, and the sex ratios of individual broods may vary dramatically with brood size^{2-4, 6-8}.

In his paper in this issue⁵, Schwarz describes a situation in which female fitness appears to increase with the number of female offspring in broods of the primitively social allodapine bee *Exoneura bicolor*. All broods are female-biased, small ones most of all, in agreement with the idea that female nest mates experience local resource enhancement (LRE). Here the interaction among siblings of like sex is cooperative rather than competitive, but otherwise LRE looks very much like an inverted form of its competitive analogue local resource competition (LRC), which is expected to produce a male bias where female siblings compete more strongly for resources than do males. This apparent symmetry is deceptive, however, because cooperative interactions among siblings can lead to outcomes of sex ratios that differ qualitatively from those produced by competitive interactions.

To see why, consider the following

simple model. Suppose that all broods are of the same size, but that parents can vary the proportion (r) of sons. If there are no sex-specific interactions among siblings, all of whom simply disperse to reproduce within the population at large, then a parent's total expected fitness through sons would be directly proportional to r , and its fitness through daughters would be proportional to $1-r$. But suppose instead that sisters interact with each other, such that their summed fitness is proportional to $(1-r)^g$. Then we can represent competitive interactions such as LRC by setting $g < 1$, so that the expected fitness of each individual female decreases as the



Fitness through sons (ordinate) versus fitness through daughters (abscissa). Top curve, $g = 1/2$; middle curve, $g = 1$; bottom curve, $g = 2$.

number of females in the brood increases. Conversely, we can represent cooperative interactions such as LRE by setting $g > 1$, so that a parent realizes increasing returns to scale on its investment in daughters.

The graph shows the combinations of fitness through daughters and fitness through sons that a parent can realize by varying r between zero (all daughters) and one (all sons), for $g = 1/2, 1$ and 2 . Because the curve for $g = 1/2$ (competition among daughters) is bowed out, a parent will achieve its greatest total fitness through daughters and sons combined by producing a mixture of both sexes; the evolutionary equilibrium (ESS) turns out to be $r = 2/3$ ($1/3$ daughters, $2/3$ sons). For $g = 1$ the curve is flat, so all brood sex ratios are equally fit if the population is in equilibrium, with overall equal investment in daughters and sons. But for $g = 2$ (cooperation among daughters) the curve is bowed in, so a parent would do better to make all daughters or all sons than it would to make a mixture of the two. Technically there is a female-biased equilibrium at $r = 1/3$ ($2/3$ daughters, $1/3$ sons), which corresponds to the male-biased equilibrium seen in the competitive case^{9,10}. But this equilibrium is dynamically unstable to

invasion by a 'split' strategy, in which with probability $1/2$ a parent makes all daughters, and with probability $1/2$ it makes all sons. If this split strategy is feasible, then the sex ratio of the whole population will be biased only slightly or not at all (depending on details of the model), and there will be a sharp division between male-biased and female-biased broods.

This is not what Schwarz finds⁵ in *E. bicolor*, which shows a strong overall female bias, with males well distributed, at least among the larger broods. If females interact cooperatively with their nest mates only during reproduction, then this distribution of brood sex ratios is puzzling. But if male offspring also benefit from being in broods that contain large numbers of females, then the problem disappears. In effect, total fitness through sons will then scale as r^h , where $h < 1$ (competitive interaction); this can easily bend the daughter-son tradeoff curve back into the bowed-out shape that favours mixed-sex broods and overall female bias. Allodapine bees develop directly into adults, rather than overwintering as larvae¹¹, so pre-reproductive females could have many opportunities to help their pre-reproductive male nest mates. Schwarz emphasizes the advantage of group defence against ants and parasites during reproduction, so it seems likely that such defence is also important before dispersal. If so, all-male broods would be at an obvious disadvantage, and the ESS could well be a brood sex-ratio distribution similar to the one actually seen in *E. bicolor*.

Nonlinear returns on investment arise in many kinds of sex-allocation problems. Why are some plants and animals hermaphrodites, whereas others are dioecious (with separate sexes)? This question seems far removed from the sex ratios of bees, and the models^{10, 12-14} proposed have not often used the language of LMC, LRC, LRE and so on. But the principles involved are seen increasingly as the same: some ecological conditions generate economies of scale in the production of one sex or kind of gamete (leading to specialization), whereas other conditions do the opposite. □

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Palaeontology

Questions of flower power

Christopher J. Cleal

THE flower is often seen as an object of beauty, but for the plant kingdom it is a source of great power. Its success as a reproductive organ is one of the main reasons why so many of the world's floras are now dominated by angiosperms. When viewed on a geological timescale, however, flowering plants have played a relatively minor role. During a total history of about 400 million years for land vascular plants, the angiosperms appeared probably no more than 200 million years ago and did not become dominant until less than 100 million years ago. How the group achieved this dominance is one of the most interesting stories in plant evolution and, on page 344 of this issue¹, Lidgard and Crane report their attempts to document the pattern of change.

Until recently, the best available evidence² was from the Potomac Group of North America, in which a significant increase in angiosperm species diversity was found in the mid-Cretaceous (about 90 million years ago). This evidence represents only a restricted geographical area and a few habitats, and does not necessarily reflect the global pattern of change. Palynological studies³ provided wider sampling and showed a similar pattern, but there remain problems. Pollen and spores are not always reliable means of distinguishing biological species, and differential rates of their distribution can bias the results.

To obtain a more comprehensive picture, Niklas and others⁴⁻⁶ statistically analysed the compositions of late Mesozoic to Tertiary macrofloras, mainly from North America. Angiosperms appeared to increase gradually in diversity during the Cretaceous, mainly at the cost of cycadophytes and pteridophytes. There are significant difficulties in interpreting this type of statistical analysis of the fossil record and, to their credit, Niklas and his co-workers discuss many of them in some detail. There is a suspicion, however, that they have made the conceptual leap from observing variations in the fossil record to assuming that this reflects global changes in floras. The validity of this leap is particularly difficult to judge as they do not document in their papers which floras they have used for their analysis (these data have to be obtained separately by writing to the authors).

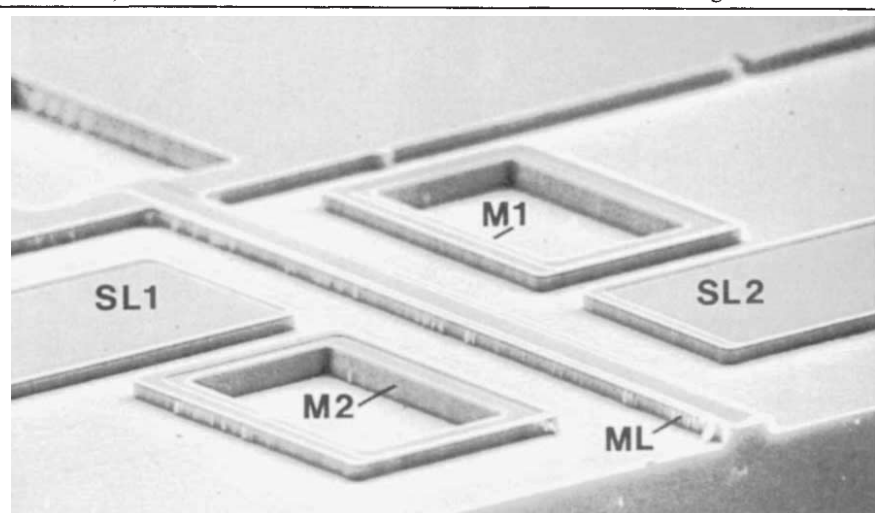
Lidgard and Crane¹ attempt to obtain more refined results by analysing the compositions of nearly 200 Northern Hemisphere macrofloras, ranging in age from late Jurassic to Palaeocene. They perform four different types of analysis to

try to overcome some of the difficulties of sampling and taxonomy. Furthermore, they only analyse leaf remains, thereby removing the problem of overestimating diversities resulting from different plant organs being given different species names. They again find that the angiosperm radiation was mainly at the cost of the cycadophytes and pteridophytes but, in contrast to the results of Niklas and co-workers, they conclude that it occurred in a sudden burst during the mid-Cretaceous.

This result seems to be an improvement on the previous studies, particularly in the use of such a large database. It is difficult to judge, however, exactly how much better it really is because details of the data used are not given in the paper (they again have to be obtained directly from the authors). There are also other weak-

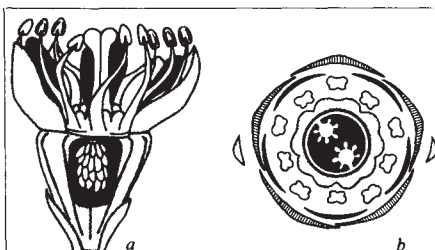
nesses in the study. As with most statistical analyses of this type, it is based on an uncritical compilation of species lists. This is particularly worrying in a study involving angiosperm leaves, which are notoriously vulnerable to taxonomic confusion⁷. It is probably inevitable considering the voluminous literature covered; and Lidgard and Crane do attempt to overcome it, both by using such a large database and by running a separate analysis on the data of W.A. Bell (ref. 8 and references therein). Nevertheless, one feels uncomfortable with a study in which the data used are potentially unreliable. Another criticism lies in Lidgard and Crane's ranking of the data into time intervals which are total artefacts: ranking of sequential data in this way has to be very carefully done, and can introduce a bias into an analysis.

But the most serious weakness lies in the absence of any palaeoecological or palaeogeographical input. Meyen has argued⁸ that the late Cretaceous change to angiosperm-dominated floras was asynchronous in different regions. It can also



LOGIC in a flash. Future computers could be based on elements like the one shown above. This optical logic gate, built by W.J. Grande and C.L. Tang (*Appl. Phys. Lett.* 51, 1780–1782; 1987) uses the 'on' and 'off' states of its semiconductor lasers as the raw material of its digital operations. Grande and Tang demonstrate that it can be used in continuous mode for the logic operations NOR, NAND and invert.

The inputs for the gate are the two side lasers consisting of an active medium (SL1, SL2) and a mirror (M1, M2). The output comes from the main laser (ML). The mechanism was first developed in the 1960s. The main laser is coupled to the two side lasers, because its active medium falls within their optical cavities. If either is on, it depletes the population inversion, necessary for laser action, in the crossing zone. For a NOR gate, either laser alone is sufficient to quench the main laser; for NAND, both need to be on. The main laser is continually powered up, and is always on unless quenched. This means that switching times depend primarily on the 'cavity lifetime' of the main laser, and could eventually be as little as 10 ps. Other proposed optical logic devices use nonlinear optical effects, requiring high laser intensities and hence high powers. Others use changes of optical wavelength or polarization and require additional external components. Grande and Tang argue that their gate uses less power, is smaller (the micrograph above is about 60 μm across; individual lasers could be as little as 20 μm long) and does not require external components, and so could be used more readily in integrated monolithic chips using current technology. Each gate could be driven, and its output detected electronically; or an entire device could be based on optical logic and communication, with the output of one gate driving the input to one or several other gates. □



Simplified reconstruction of a fossil flower from the Upper Cretaceous of Scania, Sweden. *a*, Part of ovary wall removed to show one placenta; *b*, floral diagram. (From ref. 10.)

be assumed to have been asynchronous in different habitats. Angiosperm radiation is unlikely to have been a simplistic development, as implied by Lidgard and Crane's analysis. By combining data from different regions and habitats, it might be argued that Lidgard and Crane have smoothed out this variation and provided a generalized picture of the radiation. Without a very tight control on the sampling, however, it is doubtful if any meaningful result would be produced. It would have been better for them to have tried to separate out the records from the different regions and habitats.

It is probable that a reasonably comprehensive picture of angiosperm radiation is unattainable using only palaeontological observations. The plant macrofossil record represents a very limited number of mainly lowland fluvial and lacustrine habitats. There is little direct evidence of what was happening in highland areas, deserts or other stressful areas, where the more resourceful angiosperms may have made their early advances. If the macrofossil record is to be used, the question that should be asked is not what was the pattern of angiosperm radiation, but rather how did angiosperms come to dominate the lowland, sediment-accumulating habitats? Such a question is obviously of much less general significance, but it is at least answerable with the tools now available. When viewed in this context, Lidgard and Crane have undoubtedly made an interesting contribution. It is important to remember the limitations of the study, however, and not to draw too generalized a conclusion about angiosperm evolution from the results. □

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Planetary science

Tectonic activity on Venus

Sean C. Solomon and Robert E. Grimm

AMONG the terrestrial planets, Venus is the most similar to Earth in terms of distance from the Sun, mass and bulk density. An understanding of the global tectonics of Venus could, therefore, lead to a deeper insight into the form of mantle convection and the nature of lithospheric evolution on large silicate planets. Radar images of Venus obtained from Earth-based observations and orbiting spacecraft have revealed diverse tectonic features, including analogues of terrestrial rift zones and folded mountain belts, as well as several types of complexly deformed terrain without obvious terrestrial counterparts (Fig. 1). The extent to which tectonic

hundred kilometres have been interpreted as indicating that the mantle convective-flow field beneath the equatorial highlands is dominantly one of upwelling, elevated temperatures and horizontal divergence³. All this supports the generally held view that the equatorial highlands are sites of recent or even currently active lithospheric extension and magmatism. What is disputed is the form of that extension and its relationship to the mantle flow field. What is the dominant mode of lithospheric heat transport on the planet?

J.W. Head (Brown University) proposed that Aphrodite Terra is analogous

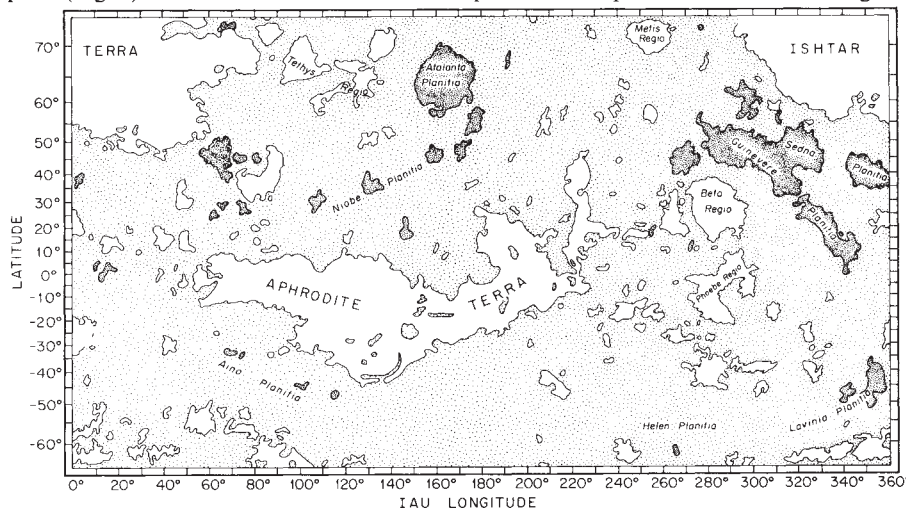


Fig. 1 Principal physiographic features on Venus¹⁰. Unshaded areas stand more than 1 km higher than the median planetary radius; darkly shaded areas, more than 1 km lower.

deformation on Venus follows regionally or globally coherent patterns and the question of whether there is plate-tectonic behaviour on Venus are issues of intense debate, which were the focus of a special session at a recent meeting*.

A key to understanding the global tectonics of Venus is the interpretation of topographic, gravity and radar imaging data from the Venus equatorial highlands, particularly the 20,000-km-long Aphrodite Terra. The equatorial highlands are associated with a nearly circumglobal system of rift valleys¹. The few well-imaged highland rift systems are flanked by extensive and relatively recent volcanic deposits and constructs². Long-wavelength variations of gravity and topography are strongly correlated on Venus, at least at low latitudes.

From these data, apparent depths of isostatic compensation (where stresses due to topographic variations are in hydrostatic equilibrium) as great as several

to a terrestrial mid-ocean ridge, where extension is accomplished by the divergence of rigid lithospheric plates and deformation is confined to narrow zones of accretion and transform faulting⁴. He and L.S. Crumpler have mapped a series of linear 'cross-strike discontinuities' (CSDs) identified from linear features and linear boundaries in Pioneer Venus radar images and topographic contours. The CSDs are oriented roughly N 20° W across the Aphrodite highland, which is elongated in a predominantly east-west direction. On the basis of high-resolution Earth-based radar altimetry profiles across the CSDs and the apparent offset of large topographic blocks by these features, Head and Crumpler hold that the CSDs in Aphrodite Terra are analogous to oceanic fracture zones on Earth. Topographic profiles across Aphrodite Terra taken in a direction parallel to the CSDs show a bilateral symmetry about *en echelon* axial segments which they argue are analogous to terrestrial spreading centres⁴.

Together with Head and Crumpler, we

*American Geophysical Union fall meeting, San Francisco, 7–11 December 1987.

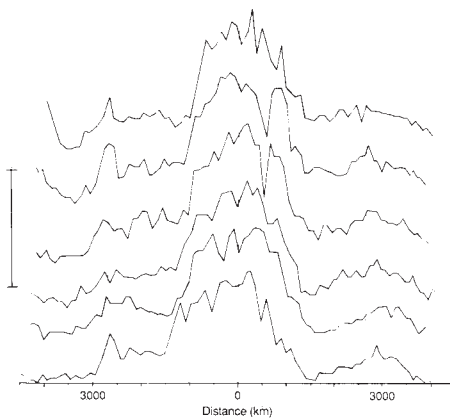


Fig. 2 Topographic profiles across Aphrodite Terra, parallel to the cross-strike discontinuities, showing the apparent bilateral symmetry identified⁴ by Head and Crumpler. Parallel profiles are ~250 km apart and cross Aphrodite near 40° E. Scale bar, 5 km height.

have carried out several quantitative tests of the plate-divergence model for Aphrodite Terra. One test, that follows from Euler's theorem for the motion of two plates on a spherical surface⁵, is whether the portions of CSDs between offset centres of topographic symmetry follow small circles about a single pole of relative plate motion. As illustrated in Figs 2, 3, these central portions of mapped CSDs pass this test, although segments distant from the axes of symmetry do not. A second test is whether the topographic profiles across axes of symmetry display a strong signature of a cooling thermal boundary layer; that is, whether elevation varies primarily as the square root of distance from the axis⁶. The fit to a boundary layer model is much poorer for Aphrodite Terra than for terrestrial mid-ocean ridges, but some profiles can be described by the model and would be consistent with apparent rates of divergence of 5–25 mm yr⁻¹. Evidently other processes such as crustal thickness variations and mantle dynamic effects contribute significantly to relief in the equatorial highlands.

Several problems with the plate-divergence model were raised. W.M. Kaula (University of California, Los Angeles) noted that the axes of topographic symmetry in the Venus equatorial highlands do not rise to a fairly uniform height above the surrounding plains, as do ridge axes on Earth. Both Kaula and R.J. Phillips (Southern Methodist University) questioned whether a plate model can be generally applied to the entire Venus surface, on the grounds that a set of continuous plate boundaries, including subduction zones, has not been identified⁶. H. Masursky (US Geological Survey) argued that the pattern of tectonic deformation in the Aphrodite region is more complex than that described by the plate-divergence model of Crumpler and Head.

Several other models have been developed to account for the topography,

gravity anomalies and deformational features of the Aphrodite region. The strong correlation at long wavelengths between topography and free-air gravity and the great apparent depth of compensation of surface relief imply that mantle convective stress couples strongly to the lithosphere⁷. Without a decoupling zone of low viscosity, mantle flow can produce lithospheric stresses in excess of 100 MPa, sufficient to induce widespread brittle failure of near-surface material. Many tectonic features on Venus could be a result of the present or past mantle flow field.

One such flow field (W.S. Kiefer, California Institute of Technology) is dominated by hot, rising mantle plumes beneath the most elevated portions of the equatorial highlands. The topography and gravity of these elevated regions are better described by axisymmetrical plume models than by a bilaterally symmetrical plate model. But the plume model does not provide a ready explanation for the linear,

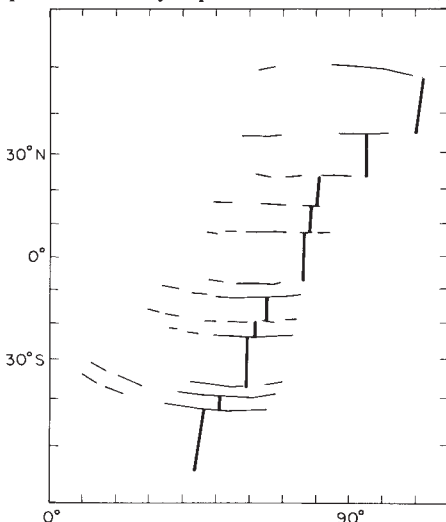


Fig. 3 Cross-strike discontinuities (faint lines) and axes of bilateral topographic symmetry (bold lines), that correspond to terrestrial mid-ocean ridges in Aphrodite Terra⁴. The plot uses a Mercator projection about the best-fitting Euler pole. If this were a region of two diverging rigid plates, the CSDs should follow lines of latitude in this projection, and axial segments lines of longitude.

en echelon pattern of rift valleys along segments of equatorial highland terrain.

A different type of convective model was offered by Kaula, who suggested that Venus could have a thick crust. Without crustal recycling, the crust would be 150 km thick if the rate of crustal formation on Venus has been similar to that of the Earth; if the thickness of the crust is limited by the depth of the basalt-eclogite transition, a value half as great could be appropriate. In either case the lower crust should be convectively unstable, although only marginally so for the lesser thickness. This line of argument leads to the idea of separately convecting layers in the crust and mantle. Because the rate of mantle heat loss will be limited by the compara-

tively sluggish convection in the crust, a low-viscosity zone should develop at the top of the mantle, favouring thermal coupling of flow in the two layers. Such a situation would be compatible with the large apparent depth of compensation of the most elevated of the equatorial highland regions. Long-term support of features with high topographic relief (mountains, volcanoes, impact craters) would be difficult in this model, but Kaula suggests that a strong concentration of heat-producing elements into the upper crust occurred on Venus, lessening the required thermal gradients in the lower crust.

According to a novel 'heat-pipe' model for volcanism and tectonics (D.L. Turcotte, Cornell University), Venus has a crust sufficiently thick for its lower reaches to be in the eclogite stability field. Episodic delamination and foundering of the eclogite layer leads to heating and remelting of the lower crust and the transport of melt to the surface. If most of the interior heat loss of Venus is transported through the crust by isolated magma conduits, the lithosphere can be sufficiently thick to support elastically the observed topographic relief. A surface volcanic flux of about 200 km³ yr⁻¹ is required. Unfortunately, such a high rate of flux is not consistent with the observed density of large impact craters on the surface of Venus⁸, which limits the volcanic flux to a figure approximately two orders of magnitude smaller⁹. Turcotte suggests that many of the large craters have a volcanic origin.

Part of the reason why no single hypothesis for the nature of crustal extension and magmatism in the Venus equatorial highlands has yet received general acceptance is that Aphrodite Terra is outside the regions of high-resolution radar imaging both by the Venera 15 and 16 spacecraft and the Arecibo Observatory. Detailed imaging of tectonic and volcanic features in the region must await the Magellan mission, due to begin acquiring global imaging data at a resolution of a few hundred metres in late 1990. Key tests of current ideas on Venus tectonics will come from the detailed mapping of deformational and volcanic features and from the determination of impact crater density and relative surface ages of the principal geological units. □

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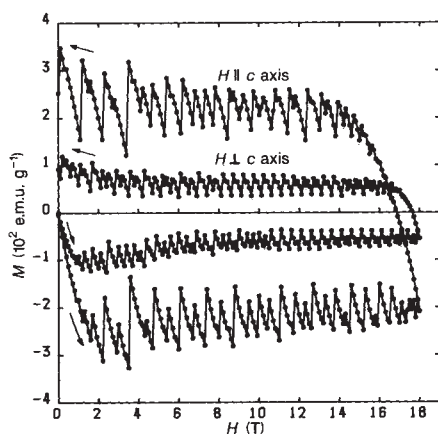
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Magnetization jumps in oxide superconductors

SIR—In your report, "New superconductors in perspective"¹, the importance of achieving large critical currents in the high-temperature oxide superconductors (HTS) was discussed for different applications. In particular, it mentioned that the critical current would be substantially improved if oriented single crystals were used instead of polycrystalline ceramics.

Besides the difficulty of having correctly oriented grains with respect to the field direction in every region of the magnet, we would like to mention here another problem we have discovered: at low temperature ($T < 15$ K), large magnetization jumps are observed in the high-field hysteresis curves of large (up to 30 mg) single crystals of $\text{YBa}_2\text{Cu}_3\text{O}_7$. This is illustrated in the figure, where the magnetization of a 27-mg crystal is shown for a field of up to 18 T applied parallel or perpendicular to the c axis.

Such magnetization jumps are not mysterious; they have been observed in conventional superconductors² and are



Magnetization curves for a single crystal of $\text{YBa}_2\text{Cu}_3\text{O}_7$ (27 mg) at 1.2 K, measured every 0.1 T up to 18 T, for a field applied parallel or perpendicular to the c axis.

attributed to the local energy flow following the movement of one or more vortex lines. Because of the low specific heat and conductivity of the HTS³, this energy flow will be transformed into a local increase in temperature, which can precipitate an avalanche of vortex-line penetration (or expulsion) — hence the jump in magnetization. These magnetization jumps look rather periodic because they take place between two envelope curves (unstable and stable magnetization) which are nearly parallel in a field far below the upper critical field H_{c2} . The amplitude of the magnetization jumps depends mainly on the sample size and on the cooling effi-

Diabetes and the potato

SIR—Jerome Rotter and Jared Diamond in their recent News and Views article¹ intriguingly predicted a higher rate of non-insulin-dependent diabetes mellitus (NIDDM) in the present-day population of Ireland by comparison with the descendants of their counterparts who left the country during the famine of 1846–51. Their rationale is that although genes predisposing to NIDDM may be deleterious when individuals have a standard westernized diet, the reverse is true under conditions of severe general malnutrition. Thus, NIDDM genes would have been favoured in the Irish who survived the famine *in situ*, and so were transmitted to subsequent generations, but not among those who emigrated.

Implicit to this line of reasoning is the assumption of a subsistence diet in Ireland before the famine, with resultant negligible selection against NIDDM genes. But all contemporary evidence contradicts this belief², largely because of the notable Irish dietary dependence on the potato.

Using the results of a detailed survey conducted by the Poor Law Commissioners in 1839, the total daily energy value of an average labourer's diet has been calculated³ as 4,720 kcal. This comprised 5.1 kg potatoes, equivalent to 1,007 g carbohydrate, supplemented with 1.8 l buttermilk. Given this diet, it seems inconceivable that there would not have been strong selection against NIDDM genes in the generations before the famine, consequently reducing their incidence in the gene pool. If a difference does exist between native-born and overseas Irish in the incidence of NIDDM, the large-scale population disturbances resulting from the famine⁴ seem to offer a more convincing explanation than differential selection.

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iciency (smaller jumps are observed in smaller samples or when the sample is in liquid helium instead of exchange gas). It depends less on the rate of field variation⁴.

The existence of these jumps strongly lowers (by at least a factor of two) the critical currents possible in large single crystals as compared with values observed in small ones. Thus, even if it is now possible to grow large crystals, it would appear necessary to stabilize them thermally (as is done in Nb–Ti wires, for example, by using bundles of small-diameter filaments), as well as trying to improve the numbers and

Optimization methods

SIR—As my recent article "New optimization methods for physics and biology"¹ has created the same unintended impression in two people's minds^{2,3}, I had better add some clarification.

I sought to bring several very different novel optimization methods to your readers' attention. These methods were developed in fields as far apart as spi-glass physics, neural-network modelling and evolutionary simulation. My sole reason for describing them in terms of the travelling salesman problem (TSP) is that this is a well-known problem familiar to many of your readers and it therefore provides a convenient common framework to expose these new ideas. I am well aware that much better algorithms exist for the TSP, as described in Johnson's letter³. Had I been writing a review of algorithms for the TSP I would have discussed these other algorithms and referred to the vast body of work by Johnson and many others.

I am interested, however, in general purpose optimization algorithms suitable for a wide range of applications. These problems may or may not be NP complete: all that is certain is that they have an unknown number of local optima in addition to the global optimum. For such systems the specialized methods developed for the TSP are not applicable. What makes simulated annealing attractive is that it is easy to implement and it often provides reasonably good solutions for diverse problems. As I pointed out¹, this is what engineers and others with practical problems to solve usually require.

May I take the opportunity to correct a more serious omission. The amount of work on genetic algorithms is much larger than I realized. Those interested in a bibliography should contact Professor David Goldberg at the Department of Engineering Mechanics, University of Alabama, Tuscaloosa, Alabama 35486.

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Deleterious mutation and genetic recombination

SIR—There are two theories of the evolution and maintenance of genetic recombination that invoke deleterious mutation: the mutation-load theory of Kondrashov¹, and Muller's ratchet². Because of the ubiquity of deleterious mutation, these theories may deserve the serious attention of evolutionists interested in recombination. So it is important to know whether the rejection of these theories by Burt and Bell³ is justified.

If deleterious mutations combine synergistically in their effects on organism fitness (that is, the marginal fitness decrement caused by the $n+1$ th deleterious mutation is greater than that caused by the n th) then a population with recombination will enjoy a lower mutation load than a population without recombination⁴. There is evidence that deleterious mutations do interact this way⁵. Therefore, as Kondrashov⁶ observed, deleterious mutation may underpin a powerful selective force favouring the evolution and maintenance of intrachromosomal recombination. But further analysis by Kondrashov¹ suggests that this force will be weak if the number of chromosomes is greater than one. This latter view was recently reiterated by Burt and Bell³ to justify their rejection of Kondrashov's theory. Kondrashov's analysis, however, is seriously misleading.

To understand why, consider the following. It is intuitively clear that alleles for recombination will be especially favoured only if they are statistically associated with the beneficial consequences of their action. For this reason, it is now common (for example, ref. 7) when modelling the evolution of recombination to suppose, quite reasonably⁸, that the allele increasing recombination is recessive and on the same chromosome where it is exerting its analysed effects. Hence the dominant alleles suppressing recombination are found on non-recombinant chromosomes and so are denied any benefits arising from the action of the recessive allele. Of course, a recombination gene may cause recombination on other chromosomes, but this fact may be ignored by modellers as the gene is unlinked to this consequence of its actions.

But in his analysis of selection at a recombination locus, Kondrashov considers only the selection arising from the effects of the locus on chromosomes other than its own. So it is only natural that he observed that selection for recombination is very weak. It is remarkable that he observed selection for recombination at all. What is more relevant to the evolution of recombination is the selection that arises from the effects of a recombination gene on its own chromosome. Much of Kondrashov's analysis of evolution in a

one-chromosome genome can logically be interpreted as addressing this question he concludes that a recessive allele causing free recombination always goes to fixation. He does not analyse the strength of selection leading to this outcome, and the qualitative considerations presented above suggest the force will be much stronger than that arising from the effects of the recombination gene on other chromosomes. So Kondrashov's theory cannot be excluded as a relevant theory to explain intrachromosomal recombination in species with many chromosomes.

The Muller's ratchet theory of recombination observes that, in a finite population of non-recombining chromosomes, the class of chromosomes with the fewest deleterious mutations may be lost by chance, and that this loss will be irrevocable. Successive losses result in a steady deterioration of the population. A population with intrachromosomal recombination, on the other hand, can restore the optimal class through recombination. In the same simulation study that led him to doubt the power of his mutation-load theory, Kondrashov observed the rapid operation of Muller's ratchet in non-recombining chromosomes.

Burt and Bell also consider Muller's ratchet to be unworthy of serious consideration as a theory of recombination although, as Charlesworth⁹ points out, it has obvious potential as an explanation of their observation¹⁰ of a positive correlation in mammals between age at maturity and 'excess chiasma number' (total chiasma number minus haploid chromosome number). Their rejection is based on Bell's previous conclusion¹¹ that Muller's ratchet is not a good explanation of the patterns of sexual and asexual reproduction in nature. This is not the place to question the adequacy of this conclusion or the understanding of Muller's ratchet. It suffices to observe that the study of Burt and Bell is relevant to the question of what forces influence intrachromosomal recombination levels in sexual species, which is a different question from what forces maintain sex.

Red Queen theories of recombination are enjoying a vogue, for good reasons. It would be unfortunate if deleterious mutation theories were ignored for poor reasons.

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Particle distinguishability

SIR—As discussed by John Maddox¹, Costantini² and Tersoff and Bayer³ feel the need to modify the traditional route to the three forms of statistics. Both papers reject the assumption of *a priori* probabilities, but for different reasons.

Costantini claims that the energy constraint in Maxwell–Boltzmann statistics destroys the equal *a priori* argument for the cells, while Tersoff and Bayer use it as a *deus ex machina* to go from classical to quantum statistics. Although Costantini is right in observing that the energy constraint does destroy the equal *a priori* probability argument, he cannot claim that the maximum of the multinomial distribution $P\{n_i\} = \{N!/n_1! \dots n_m!\} p_1^{n_1} \dots p_m^{n_m}$ gives the Maxwell–Boltzmann distribution for the n_i under particle and energy constraints because the *a priori* probabilities p_i are unknown. Boltzmann avoided this by treating the product of *a priori* probabilities as equal to m^N , which is the number of ways of permuting N particles among m cells. Note also Bernoulli's case² where the maximum of $P\{n_i\}$ is given by $n_i = Np_i$ for then $P\{n_i\} \approx 1$ when Stirling's approximation is made. The coincidence between the observed frequencies n_i/N and their expected values p_i means that the fluctuations have disappeared, and this surely can occur only in the limiting case where N and m both tend to infinity such that their ratio is constant. The fact that $p_1^{n_1} \dots p_m^{n_m}$ has been traded for the m^N in his expression for the multinomial distribution is responsible for the absence of the degeneracy factor m in his expressions (11) and (13) for Maxwell–Boltzmann statistics.

Tersoff and Bayer introduce unequal *a priori* weighting factors into the multinomial expression but their result, the inverse negative binomial coefficient, disqualifies their results, as p_i in their equation (3) does not conserve probability. There is nothing unique about their starting point as their multinomial 'distribution' is not a proper probability distribution. So their proposed 'distribution' has no closer connection to probability theory than the negative binomial coefficient itself, which is the traditional starting point for deriving Bose–Einstein statistics.

The crux of the matter lies rather in what physical situations are being considered. Take, for instance, the adsorption and de-adsorption of N molecules onto a lattice of m sites. Given that the probabilities per unit time for adsorption and de-adsorption are proportional to N and $m - N$, respectively, the solution to the one-step master equation is the binomial distribution. The particles are indistinguishable, for it makes no difference which particle is adsorbed onto which lattice site. Alternatively, when one considers Einstein's⁴ derivation of Planck's radiation law, where emission and absorption of radi-

ation are taken into account, one easily derives the negative binomial distribution as the stationary solution to the one-step master equation. Again, the particles are indistinguishable. In the appropriate limit these two distributions merge into a Poisson distribution but this distribution does not give Maxwell-Boltzmann statistics until a factor N/n is added to the entropy which tells us that the particles are distinguishable. There is nothing classical or non-classical about these distributions; merely the physical settings are different.

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More on avoiding jet lag

SIR—I believe airlines could confidently re-structure their operation to minimize jet lag. For many years passengers have suffered more than they need.

The problem lies in the convention of maintaining port of departure times during a flight. Only after landing are passengers reminded of the time difference and it is from this moment that their acclimatization begins. I promulgate a different approach which I have used for years, as it largely eliminates the problem.

Crucial to the method is the anticipation of the time-zone (and the behaviour patterns with regard to meals and period of rest) of the destination rather than the country of embarkation. If the time at the destination is eight hours ahead, then the traveller's watch is to be set at the point of boarding. Thus, if dinner served after take-off roughly coincides with breakfast at the point of arrival, the passenger should think of it as breakfast. If an eastwards flight entails arrival at dawn, then you should rest through the night, even if it was not 'night' when you boarded.

The result is that you arrive with one-third of the diurnal cycle of readjustment already accomplished. In this way, I have avoided almost all the effects of jet-lag after long periods in planes. The practical findings of Mrosovsky and Salmon, so graphically described in *Nature* **330**, 372; 1987, provide experimental evidence for a similar phenomenon in animals.

Most significantly, the authors sensibly speculate whether the internal clock "may be susceptible to feedback from overt activity". Practical experience suggests this is true. Now that there is experimental evidence for the effect, perhaps I could encourage airlines to synchronize with the destination and to serve meals compatible with where their passengers are going rather than where they have been.

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Maternal terminology

SIR—In his News and Views article on chiral morphology (*Nature* **330**, 204-205; 1987), John Galloway makes an error in terminology that is frequently committed: the confusion of 'maternal inheritance' with 'maternal effect'. He describes as an example of maternal inheritance the case in which "the hand of coiling is determined not by the individual snail's own genes but by those of its mother". This should have been described as a maternal effect. Maternal inheritance (King, R.C. & Stansfield, W.D. *A Dictionary of Genetics*; Oxford University Press, 1985), in contrast, is when a particular genetic element is transmitted only through the mother — as with mitochondria, for example. An example of paternal inheritance is the transmission of the Y chromosome in mammals. I can think of no simple example of 'paternal effect', unless one would allow sporophytic self-incompatibility, in which the genotype of the anther determines the compatibility phenotype of the pollen, regardless of the pollen's haplotype. Because the physiological, genetic and evolutionary aspects of these features of heredity are quite distinct, the distinctions between them should be clearly understood.

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Two neutrino periodicities from supernova 1987A

SIR—The detection of neutrinos from the explosion of the supernova 1987A has opened up a new area of scientific study. One line of investigation has been that of Harwit *et al.*¹, who seek to find a periodicity in the arrival times of the 12 neutrinos detected by the Kamiokande experiment and the 8 detected by the IMB experiment. They claim to have found a periodicity of 8.915 ms.

In my view, there are a number of difficulties in their evaluation of the period's significance. (1) Their use of a mean square criterion only selects periods for which the arrival times cluster in phase around the arrival time of the first neutrino. A better criterion that avoids this arbitrary restriction is to use the root mean square deviation. (2) The enumeration of periods defined by their equation 6 is oversampled by a factor of 10 when compared either to their equation 5 or to period enumeration as in a Fourier transform (that is $P = T/n$, $T = t_n - t_1$, $1 < n, T/2D$; D = timing resolution). This oversampling explains why the 'good fits' for the Kamiokande data occur in 'a single time bin'. (3) The expected number of good fits will be the number of trial periods ($T/2D$) multiplied by the probability for their

threshold of acceptance (0.00485). For the Kamiokande experiment ($T = 12$, 438.8 ms, $D = 0.1$ ms) the expected number is 301, while for the IMB experiment ($T = 5,582$ ms, $D = 1$ ms) the value is 13.5. The two data sets have overlapping ranges of trial periods when $3 < n < 6,219$ in the Kamiokande data, for which $6,217 \times 0.00485 = 30$ 'good fits' are expected. Each of these 'good fits' will have a probability of 0.00485 for matching an IMB 'good fit', so the overall probability of a chance coincidence is $1 - (1.0.00485)^{30} = 14$ per cent.

These results indicate that additional 'periodicities' could be present in the observed neutrino arrival times. In fact, my period finding routines do find many periods which satisfy each data set and several which satisfy both simultaneously. My best period is one of 9.828 ms, which has r.m.s. deviations of 15 per cent and 11 per cent of a period for the Kamiokande and IMB results respectively. This is to be compared with the r.m.s. deviation of the 8.915 ms period of 18 per cent and 11 per cent respectively.

In conclusion, the 'periodicity' found by Harwit *et al.* is likely to have arisen by chance alone from random arrival times. Indeed, other periods of higher significance are to be found in the same data. So if one period is accepted, then the second period must also be accepted.

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1. Harwit, M., Biermann, P.L., Meyer, H. & Wassermann, I.M. *Nature* **328**, 503-504 (1987).

SIR—Harwit *et al.*¹ report detection of an 8.91-ms periodicity in the neutrino burst from supernova 1987A. We argue (1) that their method is biased against the phase position of the peak and therefore should not be used for periodicity searches where the signal shape is unknown, and (2) that the 5 'good fit' period values lie well within one independent Fourier step, implying that the probability of chance occurrence of the effect is 5%, a much smaller significance than the authors state.

For a pulse superimposed on a random background, the phase distance between the centre of the pulse and a reference time t_0 (in our case the first detected arrival time) lies between 0 and 1, in a distribution that tends to be uniform for large pulse duty cycles or small pulsed fraction. Only for very small duty cycles and small pulsed fractions will the distribution peak around 0. The y -test used by Harwit *et al.* is defined by the statistical variable

$$y(f) = (1/N) \sum_{i=1}^N [ft_i - \text{int}(ft_i)]^2$$

where f is the trial pulsation frequency, N is the total number of detected neutrinos,

t_i ($i=1$ to N) denotes the arrival times, and $\text{int}(x)$ is the nearest integer to x . When there are unpulsed neutrinos from the supernova or background events, the possibility that the first event is unpulsed will introduce an offset $\delta\phi$ that will drastically alter the detectability of the pulse. Identical pulse shapes which should have the same statistical significance will instead have very different values of y . This undesirable feature of the y -test is apparent in Fig. 1, which shows the probability density distribution for uniformly distributed times (a), together with the distribution in presence of a square pulse shape with a duty cycle of 25% but centered at half period (b) or at one period (c) with respect to the time t_0 .

We have reported² the detection of a 202.4-ms periodicity in the same neutrino burst. They used the Z^2_n -test³ to provide a sensitive test for a wide range of light curve shapes (De Jager⁴ reviews the sensitivity of various periodicity search methods for sparse data sets). We applied the y -test to the pulse shape found by us (Fig. 2a) and obtained the value 0.18, as large as it would be for uniformly distributed neutrino times, but this apparent loss of significance for the 202.4-ms period is solely because the pulse shape happens to be centred around phase 0.5. In contrast, the pulse shape of the 8.9-ms period (Fig. 2b) happens to be displaced to phase 0.9, giving a small $y(f)$ value. We conclude that the y -test is only useful under the specific hypothesis of no unpulsed neutrinos and a narrow pulse duty cycle. This is not the case for the result found by Harwit *et al.*

Regardless of the inadequacy of the y -test, Harwit *et al.* make an error in concluding that the probability for chance occurrence of the 8.9-ms effect in the KAMIOKANDE data is 1.9×10^{-5} on the grounds that they have found five independent 'good fits' out of 67 trials. The frequency step used was $1/135 = 7.4 \times 10^{-3}$ Hz, more than an order of magnitude smaller than one independent Fourier step, which in the case of the Kamiokande

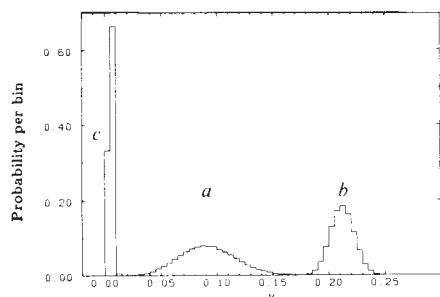


Fig 1 Probability density distribution of $y(f)$: (a) for the case of 12 events uniformly distributed in 12 s; (b) for the case of 11 events in a pulse with 25% duty cycle placed at half period with respect to the first event at $t_0 = 0$, assumed to be not related to the pulse; (c) as in (b) but with the first event time t_0 coinciding with the centre of the pulse.

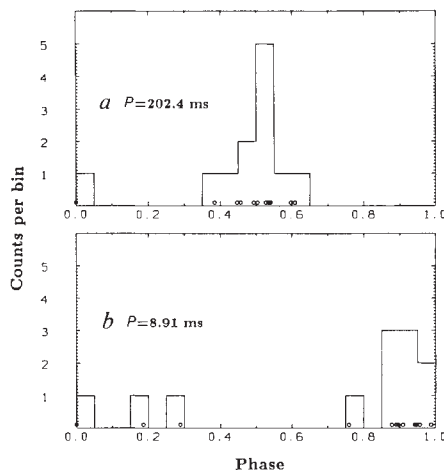


Fig 2 Light curves for the 202.4 ms (a), and (b) periods found by Ögelman and Buccheri²; and Harwit *et al.*¹ respectively. Circles indicate the phase positions of individual neutrino events.

data is $1/12.44 = 0.08$ Hz. The five 'good fits' thus refer to only one independent Fourier step and only one value should therefore be used to evaluate statistical significance. Our estimate of the chance occurrence probability of the 8.9-ms effect in the Kamiokande data, deduced from a Monte Carlo simulation that takes into account the number of frequency steps used, their spacing and the small degradation of the probability density distribution due to the low number of events, is 0.03. This value, combined with the probability of also finding 8.9-ms effect in the IMB data (quoted at 0.32 by Harwit *et al.*), gives $P = P_1 P_2 (1 - \ln P_1 P_2) = 0.05$, which is the significance of the 8.9-ms effect.

Harwit *et al.* consider as a possible explanation for this periodicity the rotation of the underlying neutron star. The birth periods of neutron stars may be very short (collapse calculations⁵ may give 8 ms, and pulsar statistics⁶ suggest 1–50 ms), but Chevalier and Emmering⁷ have shown that the pulsar statistics can also be interpreted in terms of long birth periods (90–250 ms), offering a possible explanation for the 202.4-ms period found by us.

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WASSERMAN REPLIES — Our analysis has been criticized by Schaefer, Buccheri and Ögelman on two grounds. First, they point out that we oversampled in testing our 'target set' of IMB candidate periods in the Kamiokande data. This is indeed true, an unfortunate careless error on our part. Rather than the spectacularly low probabilities of coincidence quoted in our paper, one should find agreement several per cent of the time, as noted by our critics. Second, Schaefer, Buccheri and Ögelman feel that our period search technique itself was inappropriate. Here I find their arguments less convincing, although they quite correctly point out that we have required 'periodic emission' to imply that all arrival times cluster tightly about zero phase. This was a subjective, generally restrictive choice of which our critics clearly disapprove. Personally, I stick by our opening statement that "searching for periods in sparse sets of data is a subjective enterprise". That three different ways of testing for periodicities yield discrepant results merely reinforces that view. Moreover, as we discussed, strictly periodic, narrowly pulsed emission is clearly an overly simple model. Realistic beaming patterns, for example from a surface hot spot, would imply relatively large duty cycles. The substantial mass loss due to escaping neutrinos could also cause period changes $\Delta P/P \geq 10$ per cent over the duration of the emission. Timing models that are sophisticated enough to include these effects should yield plenty of 'good fits', but at a low level of significance. Including an unpulsed component of emission would further complicate the model, adding more parameters and consequently diminishing the number of remaining degrees of freedom in the data. Moreover, it should be borne in mind that the reality of individual neutrino detections may be suspect, so that in testing models seriously one should probably account for background events unrelated to SN1987A.

Finally, observations may already be used to strongly limit possible spin periods for any still-veiled pulsar inside SN1987A. To the extent that the SN1987A light curve is well-fitted by models fed by radioactive ^{56}Co decay, constraints on the extra pulsar-driven emission (J.P. Ostriker, *Nature* **327**, 287; 1987) may be established. As reported at the recent George Mason Conference, implied pulsar periods must exceed ~ 10 ms, ruling out periods as low as 8.91 ms.

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Continental connections

Richard Bradley

Archaeology and Language: The Puzzle of Indo-European Origins. By Colin Renfrew. Jonathan Cape, London/Cambridge University Press, New York: 1987. Pp.346. £16, \$29.95.

THE popular conception of archaeology is that it derives its momentum from dramatic discoveries in the field. The reality is very different. Progress in archaeology, as in other subjects, is more deliberate but can be equally startling. The major advances happen when one of its practitioners breaks rank. An orthodoxy is suddenly held up to the light, or a problem which has been politely ignored for generations is brought back into debate.

Colin Renfrew is adept at challenging orthodoxies. Much of his early work attacked the prevailing chronology of prehistoric studies and the ways in which that sequence had been explained. More recently he has turned his attention to reconstruction, but here he takes a different tack entirely. This challenging book reminds us of crucial issues that have received little attention for 50 years.

Why is it that languages in distant areas have so much in common with one another? Why do similar features unite Celtic and Sanskrit, Armenian, German and Greek? What had India and Europe in common? And when? For earlier generations of scholars these would have been easy questions to answer, and the answers would have formed just part of a wider framework which peopled the prehistoric world with wave upon wave of migrants and nomadic invaders. It was this constant movement of population that explained anything unexpected in the archaeological record. In the traditional framework, the spread of different languages would be only one element in the complex web of relationships that seemed to account for the main developments in European prehistory.

Renfrew embarks from two premises. As his own work has done so much to show, the chronological and theoretical assumptions of the diffusionist school have very little to support them. Before radiocarbon dating developed, it was inevitable that questions of chronology would be fatally intertwined with questions of explanation. That is no longer acceptable, and more sophisticated studies of smaller regions, supplemented by an absolute chronology, have done much to emphasize the continuity of local social and economic developments. At the same time, specialists in historical linguistics still held on to what they believed was the archaeological orthodoxy. The result was a failure of communication between

professionals working in two complex but widely separated disciplines. Just as Renfrew in his earlier book *Before Civilization* took on the task of reconciling European prehistory with developments in scientific dating, he now attempts to marry the linguistic evidence with recent advances in archaeological interpretation.

No longer does it seem as if mass migration was the main agent in the spread of the Indo-European languages. Those nomads who were believed to have carried their local languages from India to Iberia

invasion hypothesis could also be documented in more detail, especially for those who are unfamiliar with archaeological research. There are other areas where Renfrew does not go far enough. In particular, he says very little about attempts to relate the spread of early agriculture to the present-day distribution of blood groups in Europe. He is uncharacteristically reticent on this point, but we do need to explore the ways in which his thesis can be tested.

In some ways this book is a summation of Renfrew's work to date. It is a sober reassessment of one of the key elements of the diffusionist framework, using recent developments in absolute dating to the full and placing a proper emphasis on the distinctiveness of prehistoric society. It employs many concepts developed in his earlier work, but it is much more than a collage of existing conclusions. It is a fresh and exciting piece of scholarship, which

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

Breaking the code — tablets from Knossos in Linear B, an early form of Greek of the Indo-European family, which was first deciphered in the 1950s.

have expired on the operating table. The Beaker Folk, once credited with so many innovations from metal-working to archery, have also gone the way of all flesh. Always more prominent in death than they were in life, they are represented by little more than a series of status symbols shared between élites over much of Western Europe. Renfrew's solution to the Indo-European problem is deceptively simple. The spread of Indo-European languages from Anatolia across three continents came with the adoption of agriculture and the gradual but inevitable population growth that then took place as larger areas of land were colonized.

The argument is lively and lucid, and the book deserves a wide readership among specialists and non-specialists alike. It is a daring thesis and at times it seems to be too much to put in one volume. There are areas where fuller discussion is called for, in particular the contribution made by local populations to the early development of agriculture. The collapse of the

goes far to rebut the charge that modern archaeologists no longer write major works of history, and is a worthy successor to *Before Civilization*. Like that book (also published by Cape) it should be issued in paperback.

Archaeology and Language addresses issues that have been studiously ignored by most of us since the early work of Gordon Childe. Like Childe, Renfrew is not afraid of dealing with big problems, and for that reason the subjects that they have discussed have much in common. But this is not a work of backward-looking piety; it is an attempt to move archaeology forward and to break its isolation. It does not matter so much whether Renfrew has given us the right solution to the Indo-European problem — though I suspect that he has — for he has started another of those debates on which progress in archaeology depends. □

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Forces in opposing corners

Richard Law

The Niche in Competition and Evolution. By Wallace Arthur. Wiley: 1987. Pp.175. £22.50, \$47.50.

THE niche is a powerful metaphor in ecology. Widely used as an architectural term, it was adapted for use in an abstract figurative sense to describe the position occupied by a person, and adapted again by ecologists to describe the role of an organism in the natural community in which the organism lives. The trouble is that because the niche was originally no more than a rather vague metaphor, ecologists have found it difficult to define, difficult to measure and even more difficult to decide whether it matters in the organization of nature.

Ten years ago, there was a consensus that interspecific competition prevented species with similar niches from living together, and that the niche played a central part in sorting the natural world into groups of compatible species. But with theoretical developments and the accumulation of further evidence from the field, this view has come under serious attack.

Wallace Arthur, who has thought more about the issues involved than most biologists, describes his own individualistic views on the role of the niche in competition and evolution in this book. First, he is a strong advocate of the experimental analysis of competition, in which populations of two species are placed in arenas and left to 'fight' for a limited supply of food. It is under these closely controlled conditions that we are most likely to see exactly what it is that enables some species-pairs to coexist, when others do not. Secondly, his leaning towards competition as a force in evolution has caused him to highlight the parallels between ecologists' interests in coexistence of species on the one hand, and geneticists' interests in the coexistence of genes within populations on the other.

This dual emphasis is strongly in evidence in the three main sections into which the book is divided. Arthur begins with an analysis of the conditions under which one species (or a gene) is replaced by another, amalgamating some basic theory with data from experimental and natural populations.

The core of the book is the section in which he describes how competition can lead to equilibria, enabling species (or genes) to coexist indefinitely. Grappling with the problem of testing whether differences in niches are responsible for coexistence, the author comes up with some

useful ideas for experimental design. His discussion of the semantic knots emanating from Gause's competitive exclusion principle, and his own resolution of the problem, also makes interesting reading.

In the final section, Arthur moves into some of the more speculative areas of competition that are currently under debate. His conclusion about the kind of structure which competition imposes on guilds of species — that their niches have to differ by more than some (variable) lower limit — is reasonable, but it is certainly not going to be easy to test. Surprisingly, he also concludes that coevolution of competing species occurs, in spite of the almost complete absence of strong supporting evidence from niche studies.

The Niche in Competition and Evolution is very much one person's viewpoint, and it should not be read as a general appraisal of the subject. Its personal style may not be to the taste of some readers, but the author is quite candid about the point at which his opinions take over from the information provided. This is a book that provokes thought and sometimes disension — and it is none the worse for doing so. □

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Soviet development

Matthew Kaufman

Cytogenetics of Mammalian Embryonic Development. By A.P. Dyban and V.S. Baranov. Clarendon: 1987. Pp.362. £35, \$75.

READING the preface to this book, one cannot fail to sympathize with the reasons why these two eminent Soviet scientists have gone to the trouble of translating and updating the Russian edition of their monograph, published in 1978. They draw attention to the fact that, to keep abreast of the world literature, most of which is published in English, our Soviet colleagues have of necessity to be multilingual, though for convenience most of their own findings appear in the appropriate Soviet journals. Unfortunately, most Western scientists peruse the Soviet journals very rarely, and even then only in translation. As is evident from the briefest perusal of the book, much excellent and original work in experimental embryology has simply passed unnoticed (by Western scientists) into the Soviet literature.

This is an unsatisfactory state of affairs, and it can only be hoped that now attention has been focused on the



Deep breathing — the first portable respirometer, designed by Zuntz and co-workers in 1906. The open-circuit apparatus made possible the long-term measurement of energy expenditure during a range of activities. The equipment consisted of a mask with manually-operated taps, a dry gasmeter and a mercury tonometer for collection of expired air samples, and was the forerunner of the Max Planck respirometer. The picture is taken from *Animal and Human Calorimetry* by J. A. McLean and G. Tobin, a survey of the theory and techniques of calorimetry as applied to the study of energy metabolism in humans and animals. The book is just published by Cambridge University Press and costs £37.50, \$69.50.

"imbalance of information exchange" (to quote the authors), those of us in the West will make more effort to familiarize ourselves with the work being carried out in the Soviet Union. It is always most depressing to come across a paper that purports to describe as new, findings which the reader himself published some years before and which the authors have omitted to cite — how much more galling it must be for our Soviet counterparts when virtually all of their work is ignored for reasons largely beyond their control.

All the effort that has been put into the production of this monograph has without doubt been time well spent. Dyban and Baranov range across a great many topics, many of which have already been covered in recent reviews and monographs. But this does little to detract from the book's value. Here are two scientists who have clearly played a central role in the establishment and nurturing of the relatively new field of mammalian developmental cytogenetics, in which the techniques used by the experimental embryologist and teratologist can be combined with those of the cytogeneticist in order to investigate the largely genetic factors that influence early mammalian development.

In the introductory chapters, the authors describe the spontaneous frequency, mechanisms of induction and the influence of haploidy, triploidy and tetraploidy on early mammalian development. The spontaneous occurrence and experimental means of inducing numerical and structural aberrations involving individual chromosomes are then discussed, with a detailed consideration of the effect of autosomal monosomy, trisomy and nullisomy, and of sex chromosome aberrations. The value and limitations of animal models as a means of studying human chromosomal anomalies are then briefly considered. In the final chapters, the authors deal in more general terms with how chromosomes and their constituent genes may control early embryonic development. Almost all of the chapters end with a 'conclusions' section, which clearly and succinctly summarizes the arguments put forward, and provides a valuable précis of the chapter contents.

The book is well written and excellently produced. It contains over one thousand carefully selected references, many of the titles of which (in translation from the Russian) indicate to me that we can have no possible excuse for ignoring the research concerned. Dyban and Baranov have done great service in providing an updated English translation of their monograph, not only for Soviet science, but for all scientists in this rapidly expanding field. □

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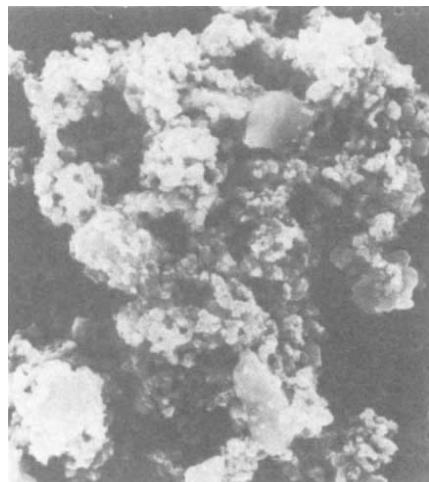
Time travelling

Steven M. Stanley

Oasis in Space: Earth History from the Beginning. By Preston Cloud. W.W. Norton: 1988. Pp. 508. \$29.95. To be published in Britain on 23 March, £19.95.

"THIS book tells the story of Earth's history from the time our Sun caught fire until now." With this opening line, Preston Cloud takes readers on an epic voyage through 4.6 billion years of time. And a delightful trip it is.

Over the years, Cloud has favoured use of the term 'geobiology' as a label for the study of life on a geological scale of time — a term that complements geochemistry and geophysics. In truth, the label has failed to take hold in the Earth science community for the same reason that for more than a hundred million years the mammals failed to expand beyond the size of a domestic cat: something else was



Stardust — a cluster of grains of cometary origin, less than 0.001 mm in diameter. First seen as a Nature cover in February 1983.

there first. Dinosaurs suppressed early mammals. Palaeobiology has suppressed geobiology. Still, Cloud's approach to research has paralleled that of the geochemists and geophysicists, in that he has pondered biotic matters on a global scale — as in his championing of the hypothesis that the gradual build-up of atmospheric oxygen triggered the evolution of multicellular animals. For this reason, he has probably been the consummate geobiologist, and this book reflects his broad scope and eclecticism.

Oasis in Space differs from most treatments of Earth history in that fully two-thirds of its pages are devoted to the interval informally known as the Precambrian — the vast stretch of time that preceded the great diversification of multicellular animals. This emphasis reflects the fact that Cloud has devoted more than two decades of his career to the Precambrian, but it is also true, as Cloud maintains, that

too little attention is customarily paid to the early history of the Earth. Accordingly, he begins with the Big Bang and offers an excellent summary of planetary origins. Next he shows how geologists read the rocks — how they date them and interpret their origins; here his discussion of the nature of time is especially intriguing. And finally, he traces the history of the Earth and its biota, with digressions to introduce the elements of plate tectonics and evolution.

The theme throughout is the interplay between physical, chemical and biological processes. This theme has merit, but I doubt that Cloud's heavy stress on the interval before the Cambrian will take hold in the general study or teaching of Earth history. The history of life becomes more interesting to most people after higher plants and animals are on the scene, and plate tectonics, which during the past 25 years has profoundly transformed our view of Earth history, must be elucidated primarily through the study of young rocks.

Anyone will find a few matters to quibble over in a book of this scope. Cloud uses the term 'eugeoclinal', which many of us would view as little more than a surrogate for the outmoded term 'eugeosynclinal', and the modern synthesis of evolution, taking its name from the title of Julian Huxley's book of 1942, preceded the Princeton conference of 1947 to which Cloud traces its origin.

Still, the information dispensed is remarkably accurate and up to date, and Cloud can turn a phrase. Wit enlivens his story, and metaphors and similes abound: "The later Archean (or Pilbaran) was like a hyperactive child — expending lots of energy with little clearly directed progress" and "Microbial heterotrophs were the hunter-gatherers of their time". I also encountered many interesting historical anecdotes that had previously escaped my attention — and a number of important facts. How many geologists know that the magnetic field of the Sun, like that of the Earth, reverses its polarity on occasion and did so between 1953 and 1958? How many know why gold is most abundant in Archaean rocks? Thus, there is much in this book to enlighten geologists whose main interest is the most recent 600 million years of Earth history. Although the book skims rather lightly over this latter interval, it does hit the high points.

Cloud's lively narrative makes Earth history accessible to the lay reader, but the book's coverage also ranges beyond the expertise of most Earth or space scientists. I commend it to them as well as to all others wishing to learn more about their geological heritage. □

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American rock

Euan Nisbet

Regional Stratigraphy of North America. By William J. Frazier and David R. Schwimmer. Plenum: 1987. Pp. 719. \$110 (North America), \$132 (elsewhere).

How does one describe an entire continent? There are two ways. One way is to skip across the land, describing only the interesting bits and broadly dismissing the rest with a few scathing comments — "We visited Europe and saw the Queen, the Botticellis and Athens: over the rest it always rains or there is smog". On the other hand, it is possible to take a deep breath, gather information for decades and then produce a vast but inevitably very much dated compilation — "Europe stretches from Ireland, soon to be given home rule if Mr Gladstone succeeds in his legislative plan . . . to [700 pages later] . . . Constantinople, the seat of the Sublime Porte which rules the Turkish Empire". Frazier and Schwimmer have chosen the latter approach. Theirs is an enormous compilation of assorted stratigraphic minutiae which, collectively, describe the fabric of the geology of North America.

The book ploughs steadily through the history of the continent, from the early Archaean to the present. The treatment of the Archaean and Proterozoic is brief: less than a hundred pages, despite the temporal span and geographical importance of these eons. But this is mostly a Phanerozoic book. As such it is an extraordinary compilation, with the crucial and critical proviso that it is a summary of the geological literature as it stood in about 1980 or 1981. Perhaps it is impossible for so vast a work to be more up to date: no doubt writing it was rather like painting the Forth Bridge — once one end is done the other is already beginning to rust again.

All the familiar stratigraphy is here, from our own old favourites in the Wiliston basin to a splendid collection of oddities across the land. There's a whole indigestible meal of formations, from the Pumpnickel to the Pepper and the Coffee (and of course the Shoo Fly), which give rise, naturally, to the Belcher and the Two Medicine formations to undo the damage. Should they prove inadequate or induce nightmares of a bestiary of Wild Cow and Womble formations, solace is no doubt to be had from the wisdom of the Zoroaster Granite or an appeal via the mercy of the Vishnu and Brahma Schists to be allowed the ultimate nirvana of the Zilch Formation. Details, details, details, all listed in a fine set of indexes and an appendix. Perhaps the closest comparison is with the Geological Survey of Canada's fine but aged compilation of Canadian

geology. Sadly, there is no discussion whatever of the advances in the past few years in understanding basin evolution.

Yet there is a valuable place for this book. Most North American geology students, struggling to pass the "Historical Geology of North America" with a decent B-, will be delighted with it, and with its innumerable tables, columns and helpful palaeontological details. Not for them to soar the heights of speculation (and immediately to be shot down) but rather the commonplace of drilling holes in the Kaskaskia. Should they wish to know in detail the lateral equivalent of the Duperow Formation they will undoubtedly have some local up-to-date paper, but it would be helpful to keep Frazier and Schwimmer in the exploration trailer anyway. Out of date and expensive the book may be, but it's still well worth having. □

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Pursuing annual subscriptions

Anthony Watkinson

Journal Publishing: Principles and Practice. By Gillian Page, Robert Campbell and Jack Meadows. Butterworths: 1987. Pp. 184. £27, \$79.95.

For scientists, formal publication of the results of their research is as important as carrying out the research itself. In most disciplines the refereed paper remains the main mode of communication. Most scholars also do their time on editorial boards, and in spite of the cuts in science budgets in many countries, and the commercialization of research throughout the world, refereeing the work of one's peers is still seen as an obligation to the community.

Nevertheless it is the common experience of publishers that the process of compiling, producing and selling journals is little understood. Even journal editors of long standing are sometimes profoundly ignorant about what, from the point of view of the publisher, are key aspects of the operation — for example, the fact that subscription rates have to be determined some six months before the year concerned and that page budgets must be set even earlier. Where scientists come together in committees, or as groups making decisions about journals publishing, the consequences of such lack of knowledge can be serious. Long established journals have collapsed and promising ideas have never been realized because of mistakes that could have been avoided.

The intended readership of this book is "the great many people concerned with journals publishing . . . whose experience of it is necessarily limited". It can be said, with qualifications, that in under 200 rather expensive pages, the greater part of the information that one could expect to find is available and reasonably easy to retrieve.

The authors are all British, and parochialism sometimes shows through. For example, the chapter on marketing is rather thin. Publicity people will be surprised at the lack of emphasis on getting sample copies to potential subscribers, which, in the United States, can often be achieved from a booth at the big congresses where the world's biggest market is conveniently gathered together. And given the intended audience, more space should have been given to a detailed analysis of contracts between learned societies and publishing companies or (as sometimes happens these days) other societies with publishing arms.

The book has probably suffered by the circumstances of its writing. It is no secret that the main author ended up doing most of the work, as is often the case in such collaborations, and the plea in the preface for feedback to enable the next edition to be better is a genuine one. By its nature the book was not going to be exciting, but the presentation does not help. The lines are too long for the print size, and the style of writing is on the dry side with an overabundance of colons and semi-colons.

For all that, *Journal Publishing* will be the standard reference book in its field because of the thoroughly commonsensical way in which it is written. It explains what is actually done, and why, and electronic publishing in all its meanings is kept in its place. So many books about aspects of publishing are written by those who would wish things to be other than they are. □

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New in paperback

- *Memoir of a Thinking Radish* by Peter Medawar, who died last year. Publisher is Oxford University Press, price is £4.95. For review see *Nature* 320, 647 (1986).
- *The Dilemmas of an Upright Man: Max Planck as Spokesman for German Science* by J. L. Heilbron. Publisher is University of California Press, price is \$7.95, £4.50. For review see *Nature* 324, 190 (1986).
- *The Mediterranean Was a Desert: A Voyage of the Glomar Challenger* by Kenneth J. Hsu, an informal account of a deep-sea drilling cruise. Publisher is Princeton University Press, price is \$8.95, £5.60. For review see *Nature* 306, 507 (1983).
- *Detectors for Particle Radiation* by Konrad Kleinknecht. Publisher is Cambridge University Press, price is £10.95, \$19.95. For review see *Nature* 325, 586 (1987).

Purification and reconstitution of the calcium release channel from skeletal muscle

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The calcium release channel from rabbit muscle sarcoplasmic reticulum (SR) has been purified and reconstituted as a functional unit in lipid bilayers. Electron microscopy reveals the four-leaf clover structure previously described for the 'feet' that span the transverse tubule (T)-SR junction. Ca^{2+} release from the SR induced by T-system depolarization during excitation-contraction coupling in muscle may thus be effected through a direct association of the T-system with SR Ca^{2+} -release channels.

CONTRACTION and relaxation of skeletal muscle is regulated by the rapid release and subsequent reuptake, respectively, of Ca^{2+} ions from the SR, an intracellular membrane system. Uptake of Ca^{2+} is effected by the SR Ca^{2+} pump, or (Ca^{2+} + Mg^{2+})ATPase, whose structure and function has been extensively characterized^{1,2}. However, the identity of the SR Ca^{2+} -release channel, and the precise mechanism by which rapid Ca^{2+} release is coupled to transverse-tubule (T-) membrane depolarization, remain unresolved issues^{3,4}.

The signal that triggers Ca^{2+} release from SR is believed to be transmitted at the T-SR junctions, specialized regions where both membranes come into apposition. Electron microscopy of T-SR junctions indicates the presence of rows of regularly spaced bridging structures (feet)^{5,6}, which span the ~120-Å gap between the two membranes. On mechanical disruption of these junctions, the feet are specifically retained by heavy-vesicle fractions derived from the junctional SR^{7,8}, which also possess enriched Ca^{2+} -release activity⁸. Rapid mixing and quench studies of ion flux using purified heavy SR vesicles show that rapid Ca^{2+} release is stimulated by micromolar levels of Ca^{2+} and millimolar levels of adenine nucleotides, and inhibited by millimolar Mg^{2+} and micromolar calmodulin and ruthenium red⁹⁻¹². Single-channel recordings of the SR Ca^{2+} -release channel incorporated into planar lipid bilayers confirm these observations, and further show that this channel has a relatively high conductance (100 pS in 50 mM Ca^{2+}) for divalent cations^{13,14}.

Much attention has recently focused on the neutral plant alkaloid ryanodine, because of its specific interaction with and nanomolar affinity for the SR Ca^{2+} -release channel^{15,16}. Vesicle ion-flux studies indicating that low (<10 μM) concentrations of ryanodine lock the Ca^{2+} -release channels open^{15,17,18} have been substantiated by single-channel measurements showing that ryanodine induces a permanently open, subconductance state¹⁹. Using [³H]ryanodine as a probe of the release channel, we have purified the ryanodine receptor and find that it is a large 30S complex comprising polypeptides with identical

apparent relative molecular mass (M_r) of ~400,000 (~400K). When incorporated into planar lipid bilayers, the purified protein exhibits a Ca^{2+} conductance with pharmacological properties of the native Ca^{2+} -release channel, and negative-stain electron microscopy reveals morphological identity with the junctional feet. These results have important implications for the physiological mechanism of excitation-contraction (EC) coupling, as they indicate that the SR Ca^{2+} -release channels, which open in response to T-system depolarization, are themselves in direct association with the T-membrane.

Purification

We purified the ryanodine receptor to homogeneity using a simple one-step procedure based on molecular-size separation (Fig. 1). Using the isolation procedure described in Fig. 1, we obtained a maximal value for specifically bound [³H]ryanodine of 650 pmol mg⁻¹ protein for the purified receptor, corresponding to a purification of 43-fold versus the heavy SR membranes (Table 1) and 6,500-fold versus the whole muscle homogenate. Assuming a single ryanodine binding site per ~400K polypeptide, the maximum theoretical specific [³H]ryanodine binding activity of the purified receptor would be 2,500 pmol per mg protein. The actual experimental value obtained is a quarter (26%) of the theoretical maximum and suggests that, under our isolation conditions, there is only one ryanodine binding site per four ~400K subunits (see also Fig. 4). This observation is not attributable to dissociation of [³H]ryanodine from its receptor during isolation as recentrifugation on a second gradient gives an identical specific activity value, and binding studies indicate a dissociation half-life for the [³H]ryanodine-bound receptor of >10 days at 4 °C. In addition, post-labelling of the purified ryanodine receptor isolated in the absence of [³H]ryanodine gives a similar specific [³H]ryanodine binding activity of 540 pmol per mg protein, and Scatchard analysis shows retention of a single high-affinity site with a dissociation constant (K_D) of 4 nM (Table 2).

Photoaffinity labelling of the purified receptor and heavy SR

Table 1 Purification of ryanodine receptor

Fraction	Protein (mg)	[³ H]ryanodine binding			~400K polypeptide	
		Total activity (pmol)	Specific activity (pmol per mg protein)	Purification	Per cent protein	Purification
1. Heavy SR membranes	1.3	19.5 (100%)	15.0	1	2.2	1
2. CHAPS-solubilized heavy SR	1.25	18 (92%)	14.4	0.96	2.1	0.96
3. Sucrose gradient (Nos 11-14)	0.025	16.3 (83%)	650	43	>98	45

Summary of ryanodine receptor purification procedure as detailed in Fig. 1. Bound [³H]ryanodine was determined by filtration¹⁶ after dilution of samples with 25 vol ice-cold water. Percentage of ~400K protein was quantitated by densitometric scanning of Coomassie and silver stained SDS gels from Fig. 1b (M, S and 12). Numbers in parentheses represent per cent recovery of total [³H]ryanodine binding activity.

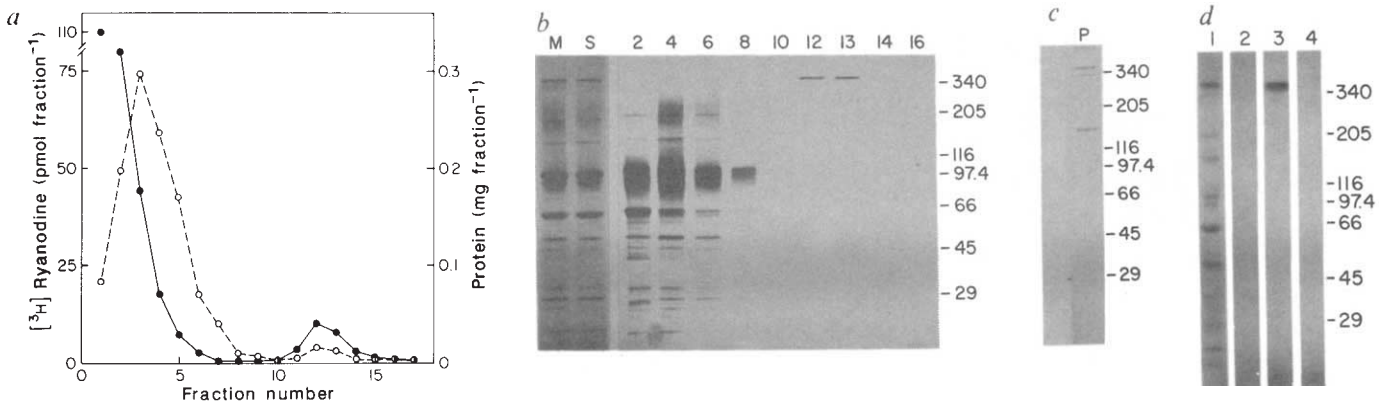


Fig. 1 Purification of the ryanodine receptor from rabbit skeletal muscle SR. *a*, [^3H]ryanodine (●) and protein sedimentation (○) profile of CHAPS (see below)-solubilized heavy SR membranes centrifuged through a linear sucrose gradient; *b*, Coomassie brilliant blue stain of SDS-PAGE of heavy SR membranes before (M) and after (S) solubilization (15 μg protein each). Silver stain of SDS-PAGE of sucrose gradient fractions 2, 4, 6, 8, 10, 12, 13, 14 and 16 (5 μl each) and *c*, partially degraded (2 days at 23 °C) gradient fraction 12 (10 μl). *d*, Fluorogram of SDS-PAGE of heavy SR membranes (lanes 1, 2) and purified ryanodine receptor (lanes 3, 4) affinity photolabelled with 8-azido-[α - ^{32}P]ATP in the absence (1, 3), and presence (2, 4) of 5 mM ATP. M_r standards (Sigma): carbonic anhydrase (29K), ovalbumin (45K), bovine serum albumin (66K), phosphorylase *b* (97.4K), β -galactosidase (116K), myosin (205K) and α_2 -macroglobulin (340K, non-reduced) are shown at the right of *b*, *c* and *d*. The subunit M_r of the ryanodine receptor has been designated $\sim 400\text{K}$ as its mobility (R_f) on 5% polyacrylamide gels (not shown) is slightly lower than that of laminin A-chain (350–400K)⁴⁴.

Methods. We isolated heavy SR vesicles from rabbit white muscle homogenates containing 0.1 pmol [^3H]ryanodine binding per mg of protein then solubilized them in a medium containing [^3H]ryanodine and the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate (CHAPS). On centrifugation through a linear sucrose gradient, a single ryanodine receptor peak was observed in the lower half of the gradient with an apparent sedimentation coefficient of 30S and corresponding to a small protein peak (*a*). All radioactivity in the top fractions of the gradient was found to be unbound [^3H]ryanodine. SDS-PAGE under reducing conditions reveals that a single $\sim 400\text{K}$ polypeptide specifically comigrates with the ryanodine receptor peak (*b*), which, when centrifuged on a second sucrose gradient produced an identical sedimentation profile and protein composition (not shown). SDS-PAGE of the purified ryanodine receptor under non-reducing conditions, and on gels of lower acrylamide concentration (5%), also produces a single band at $\sim 400\text{K}$ (not shown). On storage of the ryanodine receptor at room temperature, there is partial degradation of the $\sim 400\text{K}$ protein and appearance of two smaller polypeptides of ~ 330 and 160K (*c*). Rabbit muscle heavy SR, isolated as a 2,600–35,000g pellet²⁰ in the presence of 2.5 mM EGTA and 1 mM diisopropyl fluorophosphate (DIFP), was solubilized and labelled (1.3 mg protein ml^{-1}) for 2 h at 23 °C in buffer A (1 M NaCl, 150 μM CaCl_2 , 100 μM EGTA, 25 mM NaPIPES pH 7.1, 1 mM DIFP) containing 1.6% CHAPS, 3 mg ml^{-1} phosphatidylcholine, 2 mM AMP-PCP and 300 nM [^3H]ryanodine (2 Ci mmol^{-1}). The 100,000g supernatant (1 ml of 1.25 mg protein ml^{-1}) was loaded onto a 5–20% linear sucrose gradient in buffer A containing 0.9% CHAPS, 3 mM AMP and 4 mg ml^{-1} phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine (PC:PE:PS = 16:3:1) (Avanti Polar Lipids), then centrifuged for 16 h at 26,000 r.p.m. and 2 °C in a Beckman SW41 rotor. Gradient fractions were analysed for protein composition⁴⁵ on 5–12% polyacrylamide gradient gels, radioactivity and protein concentration⁴⁶. Apparent sedimentation coefficient of ryanodine receptor was obtained by extrapolation of an enzyme marker calibration curve ($s_{20,w}$ of 16, 11.2 and 7.6, *Escherichia coli* β -galactosidase, bovine liver catalase and yeast alcohol dehydrogenase (Sigma), respectively; $r = 0.99$). Photoaffinity labelling of the purified ryanodine receptor (isolated in the absence of [^3H]ryanodine and AMP) and heavy SR membranes, was performed with 2 μM 8-azido-[α - ^{32}P]ATP (ICN) in 50 mM NaPIPES pH 7, 1 M NaCl, 1 mM EDTA, 0.2 mM EGTA and 50 mM mannitol, by irradiating for 2 min with a 6W UVSL 58 lamp at 8 cm distance. After SDS-PAGE, gels were dried and exposed to Kodak X-ray film at -70°C .

membranes with 8-azido-[α - ^{32}P]ATP results in specific labelling of the $\sim 400\text{K}$ protein (Fig. 1*d*), indicating that a binding site for adenine nucleotides, which are known activators of the release channel^{9,11}, is retained on purification of the ryanodine receptor. Photolabelling of the $\sim 400\text{K}$ protein is adenine nucleotide-specific, as incorporation of radiolabel can be inhibited by millimolar ADP and AMP as well as ATP, but not by GTP and ITP (not shown), the last two nucleotides being relatively poor activators of SR Ca^{2+} release²⁰.

Reconstitution

We incorporated the ryanodine receptor, purified in the absence of [^3H]ryanodine, into planar lipid bilayers by fusion either of the purified soluble receptor, or of proteoliposomes containing the purified receptor. Similar results, indicating that an ion channel is present in the purified protein, were obtained in both cases, except that when we used proteoliposomes several channels are usually incorporated with each vesicle fusion into the bilayer. In both approaches, the channel is incorporated in the presence of NaCl or choline chloride, as we found that buffers containing chloride enhance the fusion rate. Figure 2*a* illustrates a single channel incorporated into the bilayer in the presence of 0.5 M NaCl and micromolar free Ca^{2+} , using the purified soluble receptor. We observed a large conductance of 595 pS under these conditions (Fig. 2*c*). We reduced the fraction of channel open time (P_0) from 0.32 to 0.03 by lowering the free Ca^{2+} concentration in the *cis* chamber (to which the purified receptor was added) from 6 to 0.02 μM . We could subsequently increase P_0 to 0.47 by adding 2 mM ATP *cis*. These observations of Ca^{2+} and adenine-nucleotide sensitivity are pharmacological characteristics previously observed for the native Ca^{2+} -release

channel from heavy SR^{13,14}. SR vesicles fuse with planar lipid bilayers such that the *cis* chamber corresponds to the cytoplasmic space and the *trans* chamber to the SR lumen^{13,14}. A unidirectional insertion of the soluble channel into the bilayer with *cis*-oriented Ca^{2+} regulatory sites also appears to be occurring, as P_0 values are unaffected by variation of the *trans* chamber free Ca^{2+} concentration. The main conducting ion is Na^+ and not Cl^- , as when NaCl is replaced with choline chloride buffer, channel conductance is reduced to background levels (not shown). We confirmed that the Na^+ conductance is occurring specifically through a Ca^{2+} channel by single-channel recordings where perfusion of the *trans* chamber with a Ca buffer and the *cis* chamber with a Tris buffer restores the conductance properties of the native Ca^{2+} -release channel (Fig. 2*b, c*). The

Table 2 Binding properties of ryanodine receptor

Fraction	K_D (nM)	B_{max} (pmol per mg protein)
1. Heavy SR membranes	6.5	15
2. CHAPS-solubilized heavy SR	4	14
3. Sucrose gradient (Nos 11–14)	4	540

Heavy SR membranes (1 mg protein ml^{-1}) were labelled in buffer A (see Fig. 1) containing 2 mM AMP-PCP and 1–10,000 nM [^3H]ryanodine for 4 h at 37 °C. CHAPS-solubilized heavy SR membranes in 4 mg ml^{-1} phospholipid (PC:PE:PS = 16:3:1) and purified ryanodine receptor were labelled as above for 10–50 h at 23 °C. Bound [^3H]ryanodine was determined by filtration¹⁶ after dilution of samples with 25 vol ice-cold water. B_{max} values of solubilized and purified samples were obtained by extrapolation to zero time using the observed half-life for receptor binding activity at 23 °C of 30 h.

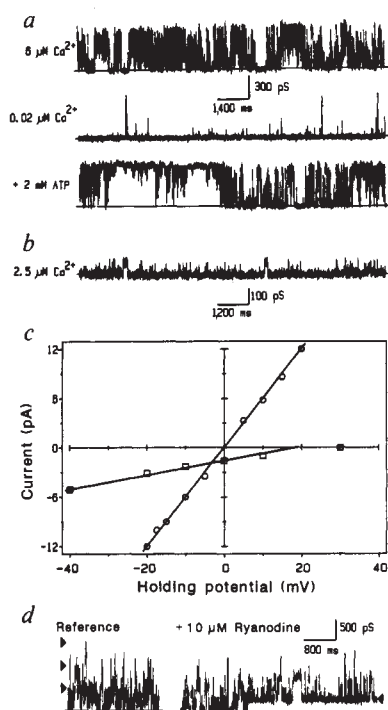


Fig. 2 Reconstitution of the solubilized, purified 30S complex into planar lipid bilayers. *a*, Single channel currents, shown as upward deflections, were recorded in symmetric 0.5 M NaCl, 20 mM NaPIPES pH 7 with 6 μM free Ca^{2+} *cis* (100 μM EGTA, 100 μM CaCl_2) (top trace), or with 0.02 μM free Ca^{2+} *cis* (2.1 mM EGTA, 100 μM CaCl_2) (second trace), or with 0.02 μM free Ca^{2+} plus 2 mM ATP in the *cis* chamber (bottom trace). Holding potential (HP), -15 mV. *b*, Single-channel current recorded after perfusion with 50 mM $\text{Ca}(\text{OH})_2/250$ mM HEPES pH 7.4, 10% glycerol *trans*, and 125 mM Tris/250 mM HEPES pH 7.4, 10% glycerol, plus 2.5 μM free Ca^{2+} (100 μM EGTA, 100 μM CaCl_2) *cis*. HP, 0 mV. *c*, Current-voltage relationship for recordings *a* (top trace) and *b*. Values of unit conductance: γ , 595 pS with 0.5 M Na^+ ($\circ$); and γ , 91 pS with 50 mM Ca^{2+} ($\square$) as the conducting ion. *d*, Multichannel recording in symmetric 0.5 M NaCl with 2 μM free Ca^{2+} plus 2 mM ATP *cis*, before (reference), and 2 min after the addition of 10 μM ryanodine *cis* (+ 10 μM ryanodine). HP, -5 mV. Left-hand arrows, three presumed current levels corresponding to three fully conducting channels of ~ 560 pS each. A ryanodine-induced subconductance state (275 pS) of one channel is indicated by the right-hand arrow.

Methods. The purified 30S complex (1–3 μl), prepared as in Fig. 1 but in the absence of [^3H]ryanodine, was added to the *cis* chamber of a planar lipid bilayer apparatus¹⁴. Channels were incorporated in symmetric 0.5 M NaCl, 20 mM NaPIPES, pH 7, and 6 μM free Ca^{2+} (100 μM EGTA, 100 μM CaCl_2) into Mueller-Rudin bilayers (PE:PS:PC = 5:3:2) formed across a 0.25 mm hole. Recordings were filtered at 300 Hz, sampled at 2 kHz and analysed as described previously¹⁴.

slope conductance of 91 pS and reversal potential of +20 mV are essentially identical to values reported for the native channel¹⁴. Moreover, in experiments where micromolar ryanodine is added to the Na^+ -conducting Ca^{2+} -channel, a reduced conductance is induced (Fig. 2*d*) which cannot then be blocked by addition of micromolar ruthenium red (not shown), a finding consistent with the recently reported effect of ryanodine on single Ca^{2+} -release channels from heavy SR vesicles¹⁹.

We also observed the properties of the native Ca^{2+} -release channel by fusion of proteoliposomes containing the purified ryanodine receptor into a planar lipid bilayer. Figure 3*a* shows a multichannel recording where, in the presence of Ca^{2+} as the permeant ion, three channels are partially activated by 10 μM free Ca^{2+} *cis*. Channel activity is increased by the addition of 2

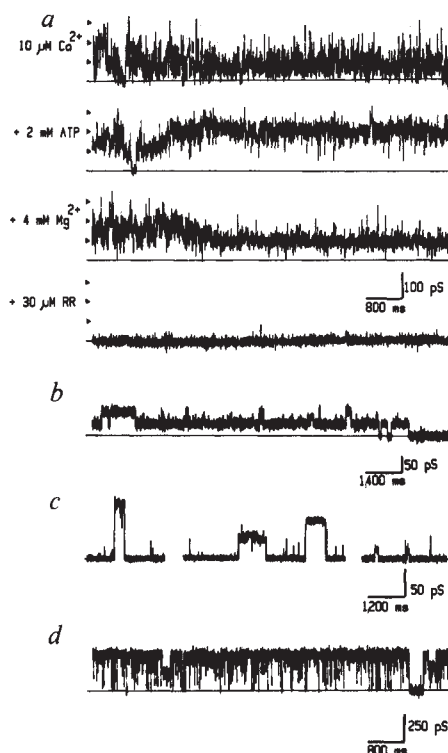


Fig. 3 Fusion of proteoliposomes containing the purified 30S complex into planar lipid bilayers and appearance of subconducting states. *a*, Three channels after reconstitution into liposomes and fusion into the bilayer were recorded with 50 mM $\text{Ca}(\text{OH})_2/250$ mM HEPES buffer pH 7.4 *trans* and 125 mM Tris/250 mM HEPES buffer pH 7.4 *cis* containing 10 μM free Ca^{2+} *cis* (top trace), plus 2 mM ATP *cis* (second trace), plus 4 mM Mg^{2+} *cis* (third trace) and after the addition of 30 μM ruthenium red *cis* (fourth trace). HP, -20 mV. *b*, Solubilized 30S complex incorporated in symmetric 0.5 M NaCl and recorded after perfusion with 10% glycerol, 50 mM $\text{Ca}(\text{OH})_2/250$ mM HEPES pH 7.4 *trans* and 10% glycerol, 125 mM Tris/250 mM HEPES pH 7.4 and 2.5 μM free Ca^{2+} *cis*. HP, -30 mV. *c*, Single-channel recording, conditions as in *a* with 10 μM free Ca^{2+} *cis* filtered at 100 Hz. HP, -80 mV. *d*, Solubilized 30S complex incorporated and recorded in symmetric 0.5 M NaCl and 6 μM free Ca^{2+} . HP, -10 mV.

Methods. The purified 30S complex, prepared as in Fig. 1 but in the absence of [^3H]ryanodine, was incorporated into liposomes by addition of 5 vol 20 mg ml^{-1} phospholipid (PC:PS = 4:1) in buffer B (0.1 M NaCl, 50 μM CaCl_2 , 10 mM TrisHCl pH 7.1) containing 1% CHAPS, and dialysis at 4 $^{\circ}\text{C}$ for 24 h against 3×1 litre of buffer B. Proteoliposomes were added to the *cis* chamber and fused into planar lipid bilayers with 51 mM CaCl_2 , 50 mM cholineCl and 10 mM TrisHEPES pH 7.4 in the *trans* chamber and 150 mM cholineCl, 1 mM CaCl_2 and 10 mM TrisHEPES pH 7.4 in the *cis* chamber. After perfusion with CaHEPES buffer *trans* and TrisHEPES buffer *cis* (*a* and *c*), channels were recorded and analysed as in Fig. 2.

mM ATP *cis* and partially inhibited by 4 mM Mg^{2+} *cis*. Opening of the channels is nearly fully inhibited by addition of 30 μM ruthenium red *cis*. The data of Figs 2*a–c* and 3*a* thus indicate that the Ca^{2+} , Mg^{2+} and adenine-nucleotide regulation sites, and conductance properties of the native Ca^{2+} -release channel, can be functionally retained by the reconstituted 30S ryanodine receptor complex.

In about half of our single-channel recordings, the P_0 is not appreciably affected by Ca^{2+} , Mg^{2+} or ATP. One noticeable difference in these recordings is the clear evidence of appearance of subconductance states. Figure 3*b* shows the presence of two Ca^{2+} -current levels, each with a conductance of 45 pS. We cannot tell whether this observation results from simultaneous incorporation of two subconducting Ca^{2+} channels, or from one channel with both a subconducting (45-pS) and a fully conduct-

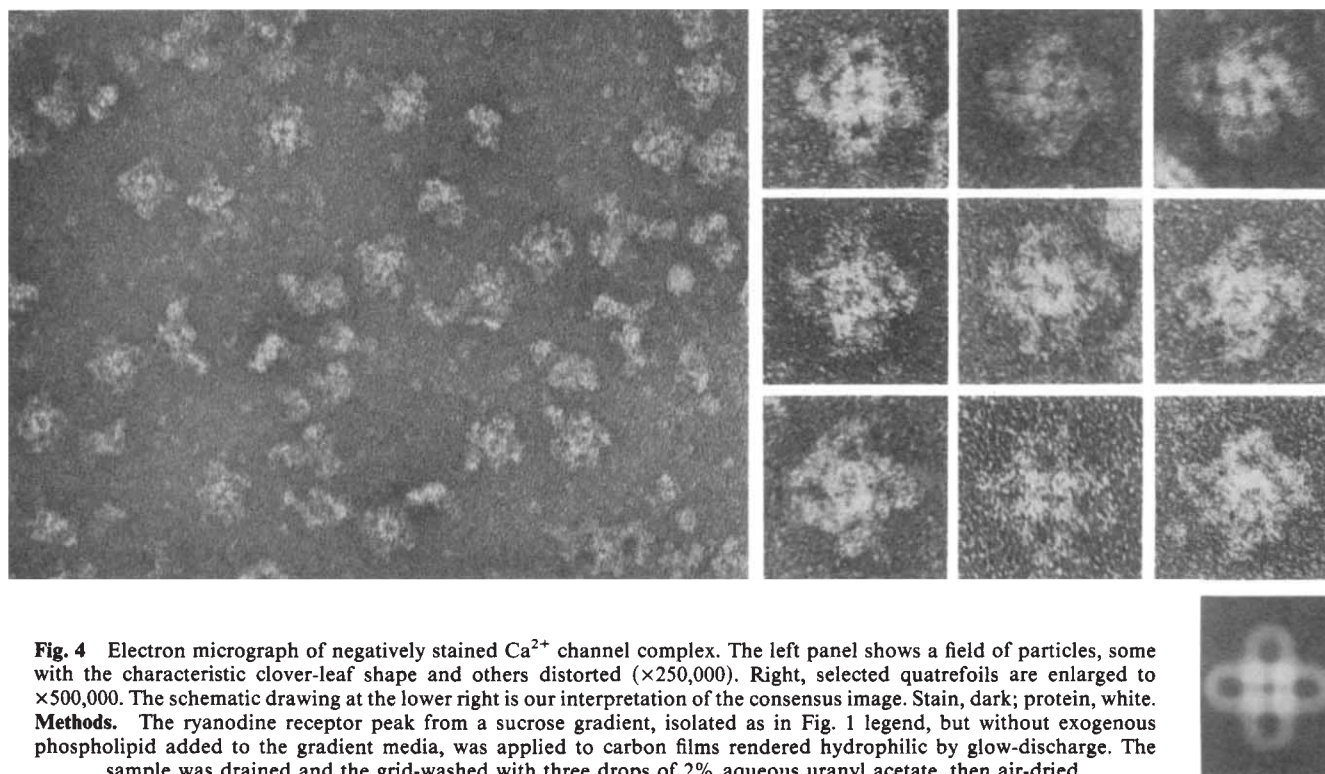


Fig. 4 Electron micrograph of negatively stained Ca^{2+} channel complex. The left panel shows a field of particles, some with the characteristic clover-leaf shape and others distorted ($\times 250,000$). Right, selected quatrefoils are enlarged to $\times 500,000$. The schematic drawing at the lower right is our interpretation of the consensus image. Stain, dark; protein, white. **Methods.** The ryanodine receptor peak from a sucrose gradient, isolated as in Fig. 1 legend, but without exogenous phospholipid added to the gradient media, was applied to carbon films rendered hydrophilic by glow-discharge. The sample was drained and the grid-washed with three drops of 2% aqueous uranyl acetate, then air-dried.

ing (90-pS) state. In another channel recording, three selected timeframes demonstrate the presence of four Ca^{2+} -current levels of 100, 65, 40 and 20 pS (Fig. 3c). We observed a similar phenomenon for the Na^{+} -conducting channel. The recording of Fig. 3d shows the presence of a subconductance state of 290 pS and occasional appearance of additional subconductances of 130 and 180 pS. These observations may result from conformational changes in a proportion of the channels during solubilization and subsequent purification which simultaneously affect their regulation and conductance properties, as those channels which retain their pharmacological profile in general do not display any subconductance states.

Structure

Electron microscopy of the purified channel complex (Fig. 4) shows four-leaf clover structures (quatrefoils) similar to those previously described for the feet²¹. For these ultrastructural studies, we purified the complex in the absence of exogenous phospholipid. Our negatively stained specimens provide higher resolution than those obtained with rotary shadowing of heavy SR vesicles²¹, which show each leaf as a round particle but with no substructure, and also show more detail than those in which the ryanodine receptor appears as a square²² or round²³ shape, with no resolution of leaves or internal details. In addition to the presence of four leaves, we observed the following details of finer structure: (1) the dimensions are 34 nm from the tip of one leaf to the tip of the opposite one, and each leaf is 14 nm wide. These dimensions are essentially the same as those reported from rotary shadowing experiments²¹. The quatrefoil could be enclosed in a square, with each leaf at a diagonal. Indeed, in many images one or more sides of a square are defined by a line of density between each leaf. The square defined in our images is 26 nm on a side, rather than the 21 nm reported previously²². (2) Each leaf seems to be a loop of protein, about 5 nm thick, around a central depression or hole 2–4 nm diameter. This could indicate that the topography of the spheres observed in shadowed images²¹ includes a deep indentation. (3) At the centre of the quatrefoil there is a region of considerably

higher protein density, ~ 14 nm in diameter, which is frequently resolved as four dense units separated by clefts. (4) The narrow clefts between the dense regions merge in the middle to form a central hole 1–2 nm in diameter.

Our earlier electron micrographs of the complex showed mostly irregular round or oval structures, about 30 nm in average diameter and 7–10 nm thick²³ (based on presumed edge-on views). We occasionally saw clear quatrefoil images in those specimens, but in our more recent preparations the quatrefoil structure is more abundant. It is not clear whether the difficulty in preserving the quatrefoil structure is at the level of protein purification or of specimen staining.

The structure of the individual $\sim 400\text{K}$ subunits and how they are distributed in the complex remains to be determined. The apparent 4-fold symmetry suggests a minimum of four subunits per complex, with a volume, assuming a 26-nm square of 8-nm thickness, sufficient to contain 4,500K of densely packed protein (at $0.73\text{ cm}^3\text{ g}^{-1}$). The 1,600K total mass of four $\sim 400\text{K}$ subunits would fill 36% of this volume, which is a reasonable estimate considering the several large clefts and depressions or holes. A mass of 1,600K would also agree with the apparent sedimentation coefficient value of 30S as S_{max} (sedimentation coefficient of an unhydrated sphere of protein) would be 49, giving a frictional ratio ($f/f_{\text{min}} (= S_{\text{max}}/S)$) of 1.63, a reasonable value for a moderately asymmetric protein. (In preliminary studies, centrifugation of the CHAPS-solubilized ryanodine receptor through linear sucrose gradients in D_2O has enabled determination of the contribution of bound detergent to the buoyancy of the complex, and we calculated a corrected sedimentation coefficient ($s_{20,w}^0$) of 35S²⁴.)

What is the location of the transmembrane segments of the channel? Although the bulk of the complex seems to lie on the cytoplasmic face of the SR in a 7–10-nm-thick planar structure^{7,21}, the channel-forming segments must project another 5–7 nm from this plane to cross the membrane bilayer. None of our edge-on views, however, show projections that could be convincingly identified as transmembrane. This is perhaps not surprising as the typical transmembrane domain of other ion-

channel subunits is $<25K^{25-27}$, which would correspond to $<7\%$ of the mass of one $\sim 400K$ subunit. Because there seems to be only a single channel per functional unit, it is tempting to place this at the centre of the complex. Thus, the high-density tetramer at the centre of the complex may correspond to a transmembrane domain from each subunit, which together form the ion-conducting channel.

Discussion

A high M_r protein doublet has previously been isolated from heavy SR and localized by immunoelectron microscopy to the T-SR junction²⁸, suggesting them to be components of the feet. In addition, the ryanodine receptor has recently been reported to comprise a protein doublet of $M_r \sim 300$ – $360K$ and a 160 – $175K$ polypeptide^{22,23}. Earlier studies indicating the susceptibility of this protein to proteolysis²⁹ are corroborated by our observation of its progressive degradation on storage, and indicates that the lower M_r components previously identified are derived from the $360K$ protein ($\sim 400K$, this study). Further, the $\sim 400K$ protein can be specifically photolabelled by calmodulin²⁹ and doxorubicin³⁰, an inhibitor¹² and activator³¹, respectively, of SR Ca^{2+} -release, and is also the sole component recognized by a monoclonal antibody capable of immunoprecipitating the [³H]ryanodine receptor³². Our findings are consistent with the above observations and show, for the first time, that the SR Ca^{2+} -release channel and all the regulatory sites of SR Ca^{2+} -release are present in a reconstituted 30S complex comprising subunits of $\sim 400K$. Thus, the feet, the ryanodine receptor and the Ca^{2+} -release channel, all seem to be synonymous.

Although the precise location of the ion-conducting pathway is not certain, the symmetry of the channel complex and the presence of a central region of higher protein density support the possibility that the ion pore is the 1–2-nm central hole formed by four membrane-spanning domains contributed by each of four $\sim 400K$ subunits. This would then be analogous to the five- and four-subunit proteins believed to form the ion-conducting channel of the nicotinic acetylcholine receptor²⁵ and GABA_A²⁶ receptor, respectively, and would agree with the ryanodine binding stoichiometry, sedimentation and structural data suggesting that the complex is a tetramer of $\sim 400K$ subunits. We cannot, however, distinguish between the possibilities that the complex is a four-subunit homo-oligomer or a hetero-oligomer, with one of the four $\sim 400K$ subunits possessing a ryanodine binding site.

The surprisingly large size of the constituent subunits and the oligomer has greatly facilitated isolation of the ryanodine recep-

tor complex, and also correlates well with its multiple functions as (1) a mechanical bridging structure across the T-SR junctional gap; and (2) a high conductance Ca^{2+} -channel with multiple recognition sites for physiological regulators. These two functions suggest a third role for the complex, which is to act as the transducer during the EC coupling process. Although physiological SR Ca^{2+} -release is triggered by an action potential believed to be communicated at T-SR junctions, the mechanism of signal transmission from the T-system to the SR remains unknown. One proposed mechanism, the Ca^{2+} -induced Ca^{2+} -release hypothesis^{33,34}, is that a small increase in cytoplasmic Ca^{2+} induces opening of the SR Ca^{2+} -release channels. The trigger Ca^{2+} released during depolarization is derived either intracellularly, or extracellularly via voltage-dependent Ca^{2+} -channels present in the T-system. Another popular hypothesis suggests that the feet directly respond to depolarization-induced, small transient intramembrane charge movements in the T-membrane^{35–37}. This leads to conformational changes of the feet which then regulate the opening of SR Ca^{2+} -release channels. Our findings, indicating that the Ca^{2+} -activated SR Ca^{2+} -release channel itself connects with the T-membrane, are compatible with both mechanisms.

Freeze-fracture studies of T-membranes^{6,38} show parallel rows of regularly arranged groups of four particles facing the parallel rows of feet. It is thus tempting to speculate that a direct relationship exists between the tetrameric feet and T-membrane particle tetramers. To elucidate the sequence of events leading to SR Ca^{2+} release, the molecular identity of these T-membrane components needs to be ascertained. Recent studies of the T-membrane dihydropyridine (DHP) receptor have shown it to comprise a 20 pS Ca^{2+} channel³⁹, although previous reports indicate that only a small fraction of DHP receptors normally function as Ca^{2+} channels⁴⁰. DHPs also simultaneously reduce intramembrane charge movements and SR Ca^{2+} release⁴¹, and the two processes are linearly related⁴². Nucleotide-sequence analysis of the complementary DNA encoding the DHP receptor shows a striking similarity to the voltage-sensitive Na^{+} channel⁴³, suggesting that it may play a dual part in EC coupling, both as a voltage sensor and as a Ca^{2+} -channel. These findings support the DHP receptor as a prime candidate for the T-membrane protein which interacts with the SR Ca^{2+} -release channel.

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The origin and location of the 5 March 1979 gamma-ray burst

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Gamma-ray bursts (GRBs) have been controversial since their discovery, mainly because of the absence of counterparts at any other wavelength that would help identify the nature of the astrophysical object associated with them; this in turn could determine their distances and luminosities, providing some constraints on their physical parameters. The absence of identifiable counterparts, as well as certain spectral features attributed to red-shifted e^+e^- annihilation radiation, has led to the conclusion that they occur on isolated neutron stars and hence their luminosities should not be far in excess of $\sim 10^{38} \text{ erg s}^{-1}$, the Eddington luminosity for a solar mass object. On this assumption, the observed fluxes of 10^{-5} – $10^{-6} \text{ erg cm}^{-2} \text{ s}^{-1}$ gave distance estimates of the order of a few kpc, although their isotropic distribution may be in conflict with such an assumption. Perhaps the most controversial GRB is that of 5 March 1979, precisely because there is an accurate positional identification of this burst¹ with a known astronomical object, the supernova remnant N49 in the Large Magellanic Cloud (LMC). Such an association would fix the distance at 55 kpc, and with the observed flux (this is by far the brightest GRB observed to date), would require prodigious energy and luminosity from this GRB (10^{44} erg and $10^{44} \text{ erg s}^{-1}$ respectively), casting doubt, on theoretical grounds, on its distance and its physical association with the LMC. However, some Kosmos 856 observations² may provide more direct evidence on energy released and hence give a decisive answer to this question.

The main criticisms raised against the LMC location of the GRB 790305 are that: (1) the luminosity greatly exceeds the Eddington luminosity for a $\sim 1 M_{\odot}$ neutron star³; (2) the magnitude of the energy radiated poses severe problems for thermonuclear explosion models, and the exceedingly short cooling time (much shorter than the duration of the burst), given the inferred luminosity, makes it difficult to supply the required energy sufficiently fast (A. G. Petschek & J. G. Colvin, preprint); (3) the opacity of γ -rays to Compton scattering and pair-production^{5,6} make the spectral appearance of the burst hard to explain. On the other hand, the LMC location is strongly favoured by statistical arguments⁷. The Eddington luminosity arguments can be easily circumvented, since this was an explosive and not a steady-state event (supernovae, for instance, also radiate well above their Eddington limit). The rest of the criticism has been rather successfully addressed in the model put forward by Ramaty *et al.*⁸ and elaborated by Ellison and Kazanas⁹, and a radiation mechanism reproducing the main spectral features of this event has been discussed in ref. 10. The basic notion of the model of refs 8 and 9 is that the energy source is gravitational, attributed to the recollapse (or major restructuring) of a neutron star in N49 following a phase transition at its centre (due perhaps to pion condensation in nuclear matter). The kinetic energy of collapse is stored mechanically in oscillations of the neutron star, which continuously feed the radiating surface with energy, thus avoiding the short cooling-time problem. As a result, the impulsive phase of the burst lasts as long as the neutron star oscillations, which damp out in $\sim 0.1 \text{ s}$ mainly through the emission of gravitational radiation¹¹, in agreement with the duration of the impulsive phase. The nature of the prolonged ($\sim 50 \text{ s}$) emission, at much lower luminosity, is less certain. It may be due to the radial oscillations^{8,9} which, immune from

gravitational radiation, damp out on much longer timescales than the non-radial ones responsible for the impulsive emission phase. The major characteristic of the prolonged emission phase is its coherent oscillatory behaviour, commonly attributed to the rotation of the neutron star. The period of rotation was found to be $P = 8.00 \pm 0.05 \text{ s}$ (ref. 12).

With this picture in mind we now turn to the observations of Kosmos 856². This satellite operated for two years before the 5 March burst and observed twice, in the scanning mode, in the direction of the N49, for $\sim 90 \text{ s}$ at a time. A period analysis with a periodogram technique (folding the data with a given period), gave in one of the scans two peaks at periods 8.6 ± 0.6 and 4.3 s , and no significant periodicities in the other. The authors noted that these were different from those of the 5 March 1979 GRB¹² but attributed the difference to statistics. They noted that if the periods are indeed different, the pulsar must have increased its spin rate by $\sim 5\%$ in two years, but did not discuss, for example, how significant their signal would have been had they folded their data with 8.05 s or 8.3 s periods. The fact that the power in their periodic signal dies out completely for $P < 8.3 \text{ s}$ suggests that their data would not have given significant periodicities at $P = 8.05 \text{ s}$ or 8.3 s , implying that the differences may not be merely statistical. We point out here that the differences appear to be more significant than the authors claim, and may have far-reaching consequences which have not been discussed at all. In particular, the period difference would indicate an absolute change of the rotational energy associated of the object of $\Delta T = I\omega\Delta\omega \approx 3 \times 10^{43} \text{ erg}$ (assuming that the moment of inertia I is constant) independent of the distance, because most neutron stars have moments of inertia $I \approx 10^{45} \text{ g cm}^2$. Such a prodigious change in the rotational energy needs to have been effected within approximately two years, and it is very difficult to see how the kinetic energy of an isolated neutron star can increase at such a rate. The increase could possibly have been achieved by accretion if the neutron star were in a binary system, but the associated energy release of approximately $10^{36} \text{ erg s}^{-1}$ would be very difficult to hide, especially if the emitting object is a foreground object in our Galaxy, relatively nearby ($d < 300 \text{ pc}$) as most theories advocating a galactic origin suggest³⁻⁶. On the other hand, the model of ref. 8 can naturally provide changes in the rotation energy of this magnitude, not over the period of two years but rather in a fraction of a second, in the very event that was responsible for the 5 March 1979 GRB. This is achieved by decreasing the moment of inertia rather than increasing the angular momentum, thus bringing into natural association the observed change in rotational energy and the GRB 790305. Detailed models of this kind have been computed by Haensel and Proszynski¹³ who estimated the changes in binding energy, internal energy and moment of inertia for neutron stars whose equations of state allow phase transitions in their interiors which require major restructuring. The change in moment of inertia depends on the details of the phase transition (the density difference between the two phases, the amount of matter that undergoes the transition and so on. Values consistent with the observations, $(\Delta I/I = \Delta\omega/\omega \approx 6 \times 10^{-2})$ are possible if the matter within $\sim 3\%$ of the radius, from the centre of the neutron star, converts into the second phase. But because the neutron star is a slow rotator (the rotational forces are not dynamically important), most of the available potential energy is converted into oscillatory and internal rather than rotational energy. Therefore, the rotational energy change $\approx 3 \times 10^{43} \text{ erg}$ is only a lower limit on the energetics involved. The efficiency for converting the oscillations into gamma rays must have been quite low^{8,9}. Most of the kinetic energy of the collapse was radiated by gravitational waves, and neutrino emission might also have been important. The gamma-ray conversion efficiency is always an unknown quantity in these models, but there is in principle enough energy available, and a small fraction of it could easily account for the energetics of the burst. There are still many questions concerning various other aspects of the

problem (see for instance a recent criticism by Liang¹⁴), which may force us to reconsider some of the particulars of this model. We do not pretend to be able to answer all these questions, but we think that our interpretation of these observations agrees with the basic premises, that is, the energetics, of the model of refs 8 and 9.

There is one more reason why resolution of the issue of the distance to the GRB 790305 is important. There appears to be a distinct emerging class of GRBs¹⁵, with only three members currently whose characteristics are similar to those of GRB 790305. They are recurrent and generally have short rise times and durations (with the exception of the oscillating phase of GRB 790305) and soft ($kT \approx 30$ keV) spectra. Because of their recurrence they became known as 'repeaters'. An additional distinct characteristic of the repeaters is their spatial distribution, which is similar to that of X-ray sources¹⁵, like them they are located in the galactic plane and the LMC. Their association with the galactic plane implies that they are distant objects ($D \approx 10$ kpc) thus hinting that the GRB 790305, a natural member of this class, is also a distant object. In addition, their soft spectra are consistent with the arguments of Kazanas and Ellison¹⁶, suggesting a correlation between the spectra and distances of GRBs. These authors argued that if there is a common emission mechanism for all GRBs, the most distant ones (which should be located mainly on the galactic plane) should have soft spectra. The reason is that, being very distant, these GRBs are the most luminous ($L \approx 10^{40}$ erg s⁻¹), and therefore they should convert all their γ -rays to an optically thick e^+e^- plasma via the $\gamma\gamma \rightarrow e^+e^-$ process. For a spherical source of luminosity L and size R , the Thomson depth of the resulting e^+e^- plasma is expected to be $\tau_{\pm} \approx 10(L_{38}/R_6)^{1/2}$ (refs 16–18), where $L_{38} =$

$L/10^{38}$ erg s⁻¹ and $R_6 = R/10^6$ cm; this plasma would then thermalize and soften the emerging photon spectrum.

The emergence of a specific class of GRBs and the correlation of spectral softness with distance to the burst have implications not only for the repeaters but also for the other GRBs¹⁶. The issue of the distance to GRB 790305 thus takes on further importance within the broader context of the nature of all GRBs, rather than the question of the nature of an odd and maybe unique event. We hope that this note will stimulate a more detailed re-evaluation of the data of ref. 2, resulting in a more satisfactory answer to this question.

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¹⁵N-enriched nitrogen in polymict ureilites and its bearing on their formation

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Three polymict ureilites have been identified as a subunit within the ureilite group of achondrites¹: North Haig, Nilpena and the Antarctic meteorite EET 83309. They are characterized by their brecciated nature and the presence of lithic clasts and mineral fragments of rock types different from main-group ureilites (MGUs). Here we present analysis of the nitrogen isotope compositions of these three ureilites, which show them to be highly enriched in ¹⁵N; measured $\delta^{15}\text{N}$ values reach +540‰ relative to air, but mass-balance calculations suggest a component of at least +600‰. Nitrogen in unbrecciated MGUs does not exhibit such unusual isotopic compositions. At least two nitrogen components exist, and analogies may be drawn with the Bencubbin meteorite (also an achondritic breccia), but no definite connection can be made. We favour a hypothesis that calls for the introduction of isotopically heavy nitrogen into polymict ureilites during a brecciation event caused by a more primitive, possibly carbonaceous chondrite-like projectile.

We have used static mass spectrometry to study the isotopic composition of nitrogen released from several ureilites by stepped combustion. (Isotopic compositions are expressed in the delta (δ) notation, as a parts per thousand (per mil, ‰) deviation from an international standard. For nitrogen, this standard is air.) Nitrogen in MGUs occurs as a mixture of components; $\delta^{15}\text{N}$ varies from -80‰ to +20‰^{2,3}. Nitrogen in polymict ureilites is different: although the components recognized in

Table 1 Results of stepped combustion of whole-rock samples of three polymict ureilites

Temp. (°C)	EET 83309			Nilpena		North Haig	
	[N] (p.p.m.)	$\delta^{15}\text{N}_{\text{air}}$ (‰)		[N] (p.p.m.)	$\delta^{15}\text{N}_{\text{air}}$ (‰)	[N] (p.p.m.)	$\delta^{15}\text{N}_{\text{air}}$ (‰)
200	25.5	+1		5.5	-1	17.7	-4
300	8.7	+288		9.5	-28	42.3	+35
400	10.7	+527		7.3	+91	30.8	+26
500	7.7	+359		4.2	+153	17.1	+38
600	7.4	+238		1.9	+137	10.7	+46
700	6.8	+127		6.8	-61	10.5	+36
800	3.8	+118		3.1	-79	10.2	+20
900	1.3	+125		0.3	+99	7.8	-0
1,000	1.1	+87		0.4	+8	1.8	+22
1,100	—	—		0.5	+31	—	—
1,200	2.1	+63		0.2	+39	3.0	+11
Total	75.1	+192		39.7	+32	151.9	+26

Yields are expressed as the concentration of nitrogen released in each 100 °C temperature interval.

MGUs may be present, the nitrogen has greatly elevated $\delta^{15}\text{N}$ values (Fig. 1a and Table 1). Isotopically heavy material is present in varying quantities, and is most apparent in EET 83309, which has the highest enrichment in ¹⁵N. In EET 83309, nitrogen with high positive $\delta^{15}\text{N}$ is liberated from room temperature to 1,200 °C, reaching a maximum of +527‰ between 300 and 400 °C, where the greatest quantity of indigenous nitrogen occurs (11 p.p.m.). As temperature increases above 400 °C, both nitrogen concentration and $\delta^{15}\text{N}$ decrease, presumably indicating that residual quantities of ¹⁵N-enriched nitrogen are liberated simultaneously with isotopically lighter nitrogen from other sources. The variation of $\delta^{15}\text{N}$ observed in Nilpena (Fig. 1b and Table 1) could be interpreted similarly as isotopically heavy

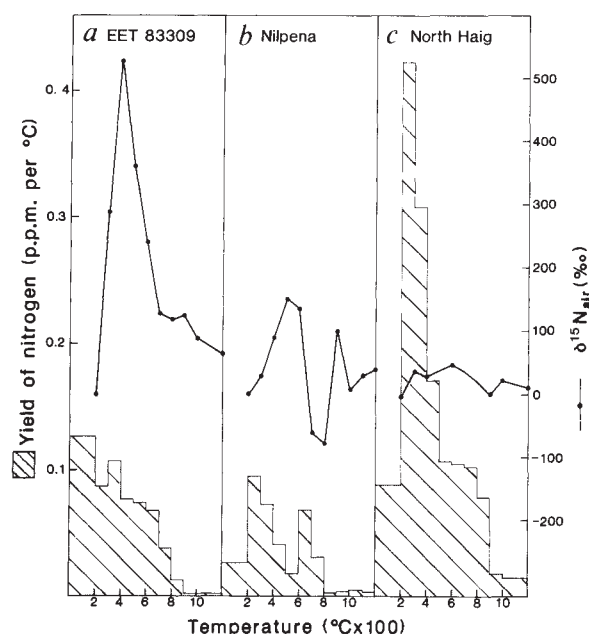


Fig. 1 Nitrogen released by stepped combustion of whole-rock samples of *a*, EET 83309 (5.304 mg); *b*, Nilpena (5.641 mg); *c*, North Haig (3.858 mg). Histograms show yield of nitrogen (p.p.m.) of whole-rock meteorite per °C; curves show $\delta^{15}\text{N}$ (‰) relative to air; 2σ error bars on $\delta^{15}\text{N}$ are less than the size of the spots.

nitrogen (300–1,200 °C), superimposed on top of which is a component of isotopically light material (600–800 °C). Assuming that atmospheric nitrogen is desorbed in the first (200 °C) temperature step, the yield of isotopically heavy nitrogen is depleted by a factor of two in Nilpena (24.3 p.p.m.), relative to EET 83309 (49.6 p.p.m.). Coupled with occurrence of the light component (9.9 p.p.m.; $\delta^{15}\text{N} < -79\%$) the decrease in the amount of isotopically heavy material serves to lower the overall $\delta^{15}\text{N}$ of Nilpena by $\sim +160\%$.

The third polymict ureilite, North Haig (investigated as part of an earlier study²), also contains nitrogen enriched in ^{15}N , with a maximum $\delta^{15}\text{N}$ of $+46\%$ and a greatly increased abundance (~ 150 p.p.m.), at least four times that of almost every other ureilite measured (Fig. 1c, Table 1 and refs 2, 3). North Haig is a badly weathered meteorite 'find', so its high nitrogen content was assigned to the breakdown of terrestrial contaminants² (for instance, ammonium salts and nitrates). This argument may still hold, but the $\delta^{15}\text{N}$ of $+46\%$ may be an indication of 5–10 p.p.m. of a component with the extreme value seen in EET 83309 showing above a background of terrestrially derived weathering products.

Despite the differences in nitrogen isotopic composition among polymict ureilites, all three samples contain nitrogen characterized by high enrichment in ^{15}N . If this component is absent from unbrecciated MGUs^{2,3}, the isotopically heavy nitrogen could be related in some way to the brecciation event. One interpretation is that it was introduced to the samples during brecciation, either from the parent-body atmosphere or from the projectile. In the search for the identification of suitable candidates for the impactor, it should be considered that significant quantities of isotopically heavy nitrogen occur in many carbonaceous chondrites^{4,5}. Isotopically heavy nitrogen also occurs in several other meteorites: as a minor component in a clast from the brecciated enstatite chondrite Abee⁶, in abundance in the achondritic breccias Bencubbin and Weatherford (in Bencubbin, almost all the element has $\delta^{15}\text{N} \approx +1,000\%$)^{7,8} and the shergottite EETA 79001⁹. As the shergottites are thought to have a unique origin on Mars and the ^{15}N -enriched nitrogen (liberated at $T > 900$ °C) is believed to represent trapped martian

atmosphere, this component is unlikely to be related to that in polymict ureilites. To assess any links with isotopically heavy nitrogen in other meteorite groups, it is desirable to place some constraints on the nitrogen carrier.

Pyrolysis of EET 83309 whole-rock (Fig. 2a and Table 2) resulted in the release of isotopically heavy nitrogen above 200 °C, with $\delta^{15}\text{N}$ rising to a maximum of $+540\%$ at 500–550 °C. The quantity of nitrogen liberated on pyrolysis and its weighted isotopic composition is similar to that obtained on combustion (64.0 p.p.m. with $\delta^{15}\text{N} \approx +191\%$ compared with 75.1 p.p.m. with $\delta^{15}\text{N} \approx +192\%$ respectively). The pyrolysis experiment was done with 50 °C temperature increments between 300 and 700 °C, and revealed a bimodal abundance pattern, but not two distinct plateaux in isotopic composition. Although the nitrogen release is better resolved on pyrolysis than on combustion, it is not yet complete.

Stepped combustion of an HF/HCl-resistant residue obtained by partial demineralization of EET 83309 (1:1 v/v 28M HF/12M HCl at 20 °C, followed by 12M HCl at 60 °C) yielded nitrogen with $\delta^{15}\text{N}$ only up to $+211\%$ (Fig. 2b and Table 2). The nitrogen concentration in the acid-residue (179 p.p.m.) when corrected for a weight loss of 90%, implies that $\sim 75\%$ of the isotopically heavy nitrogen has been lost during the acid treatment. A simple mass-balance calculation indicates that the missing nitrogen has $\delta^{15}\text{N}$ of $\sim +600\%$, assuming that the loss of isotopically heavy nitrogen during dissolution parallels that of isotopically normal nitrogen. Combined with the pyrolysis data, these measurements suggest the presence of two isotopically heavy nitrogen components, although in this case it is the variation of $\delta^{15}\text{N}$ with temperature that is bimodal. The first is liberated at lower temperatures (350–450 °C; $\delta^{15}\text{N} \approx +210\%$), the temperature interval across which the most isotopically heavy nitrogen was released during combustion of the whole-rock sample of EET 83309. It is possible that this component represents a residuum of $\delta^{15}\text{N} = +600\%$ nitrogen, co-released with a more abundant, isotopically normal, acid-insoluble entity. There is no second peak in the nitrogen release, but a second component in the acid-residue is suggested by the $\delta^{15}\text{N}$ of $\sim +178\%$ between 500 and 600 °C.

Table 2 Pyrolysis of EET 83309 and stepped combustion of Nilpena and EET 83309

Temp, (°C)	EET 83309 (WR, P)*		EET 83309 (HF/HCl, C)*		Nilpena (Clast, C)*	
	[N] (p.p.m.)	$\delta^{15}\text{N}_{\text{air}}$ (‰)	[N] (p.p.m.)	$\delta^{15}\text{N}_{\text{air}}$ (‰)	[N] (p.p.m.)	$\delta^{15}\text{N}_{\text{air}}$ (‰)
200	19.6	-18	21.8	-16	16.7	-10
300	1.1	+34	12.6	+25	31.9	+16
350	0.8	+51	9.1	+141	—	—
400	0.6	+182	22.4	+211	191.0	+61
450	1.8	+434	40.8	+177	171.7	+127
500	2.8	+536	27.0	+132	108.9	+102
550	2.9	+540	7.6	+178	47.7	+184
600	3.9	+510	5.5	+173	37.2	+213
650	2.1	+433	—	—	37.1	+188
700	5.0	+363	8.7	+94	31.4	+170
750	—	—	—	—	27.0	+161
800	8.6	+281	8.7	-15	49.1	+163
900	—	—	ND	ND	9.7	+154
1,000	8.5	+162	8.9	-94	ND	ND
1,100	—	—	—	—	61.7	+125
1,200	4.6	+3	6.3	-75	15.2	+118
1,200 (C)†	1.7	+8	—	—	—	—
Total	64.0	+191	179.4	+103	836.3	+116

* WR, whole-rock sample; P, pyrolysis; C, combustion; ND, not determined. Yields are expressed as the concentration of nitrogen released in each temperature interval (50 °C or 100 °C).

† After pyrolysis at 1,200 °C, the sample was combusted at the same temperature to determine whether all nitrogen had been removed.

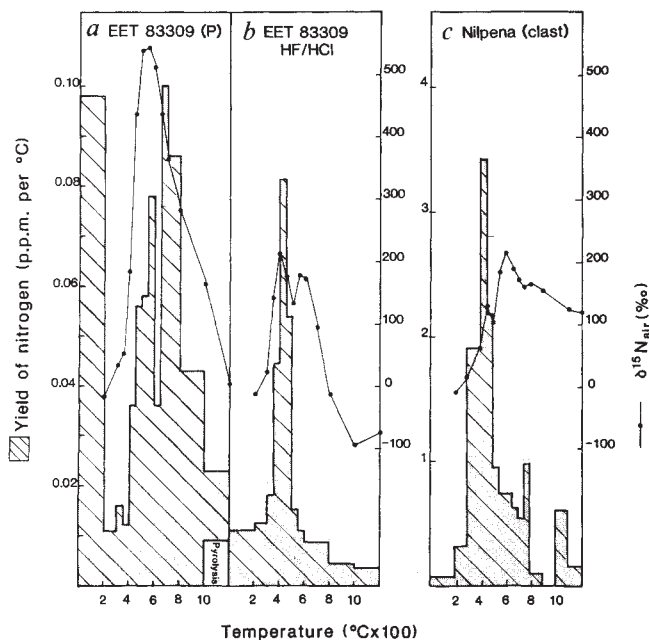


Fig. 2 Nitrogen released from *a*, EET 83309 whole-rock sample (2.365 mg, pyrolysis); *b*, EET 83309 HF/HCl-resistant residue, 90% weight loss, data normalized to whole-rock sample (0.845 mg, combustion); *c*, a carbonaceous chondrite-like clast from Nilpena (0.246 mg, combustion). Histograms show yield of nitrogen (p.p.m.) of whole-rock meteorite per °C; curves show $\delta^{15}\text{N}$ (‰) relative to air; 2σ error bars on $\delta^{15}\text{N}$ are less than the size of the spots. Note change in scale on yield axis for Nilpena.

A further specimen that may help in attempts to identify the location of isotopically heavy nitrogen in polymict ureilites is the clast from Nilpena described by Prinz *et al.*¹⁰, apparently similar to carbonaceous chondrite matrix. Stepped combustion (Fig. 2*c* and Table 2) of a portion of this material released isotopically heavy nitrogen at all temperatures >300 °C, reaching a maximum $\delta^{15}\text{N}$ of +213‰ at 500–600 °C. This $\delta^{15}\text{N}$ is greater than in the whole-rock sample of Nilpena and nitrogen yield is much higher than in all of the polymict ureilites: 836 p.p.m. Comparison of the nitrogen isotopic variation in the Nilpena clast with that in the HF/HCl residue from EET 83309 shows a great similarity between the two patterns. It appears that the acid-insoluble ^{15}N -enriched nitrogen component is concentrated in the clast material, but not the isotopically heaviest nitrogen component.

Nitrogen of unusual isotopic composition is liberated from whole-rock samples of polymict ureilites on both stepped combustion and pyrolysis, although the nitrogen release is displaced to higher temperatures by ~150 °C on pyrolysis. Combustion over 100 °C increments implied that isotopically heavy nitrogen had an extended thermal release, but a higher resolution pyrolysis experiment showed that the abundance pattern is bimodal. Data from the HF/HCl-resistant residue from EET 83309 and the clast from Nilpena, taken with the pyrolysis data, imply that two ^{15}N -enriched components are present. The first component (N_1) is acid-soluble (~75% of the heavy nitrogen is removed on acid-treatment), with $\delta^{15}\text{N} \approx +600\%$ (liberated on combus-

tion at 300–500 °C and pyrolysis at 450–600 °C), the second (N_{II}) is acid-insoluble, with $\delta^{15}\text{N} > +200\%$ and a combustion temperature of 500–700 °C (650–800 °C on pyrolysis). N_1 has the highest $\delta^{15}\text{N}$ observed for a component in a meteorite after N_α and N_β in Bencubbin⁷. There are similarities between N_1 and N_α and between N_{II} and N_β in terms of stability during pyrolysis or combustion and resistance to strong acids. Bencubbin also contains carbonaceous clasts¹¹, and Weisberg *et al.*¹² have catalogued a variety of reasons to suggest a connection between Bencubbin and ureilites in general (the same group has extended these ideas to include other meteorites¹³, one of which, Renazzo, has characteristically isotopically heavy nitrogen). Clearly, these are tentative relationships which may be circumstantial, but the isotopic similarities between polymict ureilites and Bencubbin components require more formal examination. For now, it is still possible that the ^{15}N -enriched nitrogen is unconnected with anything previously studied, and that N_1 is labile because it exists as a loosely bound trapped gas or an acid-soluble inorganic material such as a nitrate.

The apparent absence of heavy nitrogen from MGUs suggests that the ureilites as a whole were not derived from carbonaceous chondrite-like precursor material (cf. ref. 14) enriched in ^{15}N , such as Renazzo. A more attractive alternative in keeping with the MGU data is that the ^{15}N -enriched nitrogen was introduced as a solid or gaseous component by the projectile which caused brecciation. This hypothesis fits the model in which MGUs have a magmatic origin, forming deep within their parent-body and shielded from external processes such as bombardment by more primitive material (cf. ref. 15). If ^{15}N -enriched nitrogen were introduced by an external process, then the chondritic clast from Nilpena may represent the remnants of the projectile responsible for brecciation. Redistribution and subsequent isotopic fractionation among several different sites within the ureilite parent-body may account for the differences in nitrogen concentration and isotopic composition among the polymict ureilites analysed. But it will be instructive to examine clasts of the other examples of primitive meteoritic material known to occur in polymict ureilites, to place more rigorous constraints on the origin of isotopically heavy nitrogen in these brecciated meteorites.

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Molecular manipulation using a tunnelling microscope

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In a very short time the scanning tunnelling microscope has become an important tool in surface science, and physics in general¹⁻³. Its primary use has been to obtain atomic-resolution images of surfaces, but recently, efforts have been made to use it to manipulate materials as well as image them⁴⁻¹⁰. One may now reasonably ask if it is possible to move and alter matter predictably on an atomic scale. Here we report the accomplishment of the smallest yet, purposeful, spatially localized changes in matter, effected on a graphite surface. We believe that the changes result from the pinning of individual organic molecules to the graphite. The reverse manipulation, the removal of pinned molecules, has also been demonstrated. Finally, we have evidence that we can remove a portion of a pinned molecule, effectively performing transformations on single molecules using the tunnelling microscope.

The scanning tunnelling microscope (STM) consists of a conducting needle held close to a conducting surface. As the needle approaches a distance of ~ 10 Å from the surface, electrons can tunnel with a reasonable probability between the needle and surface. A bias voltage is applied between the two conductors, and the resulting current is a sensitive measure of their separation. As the tunnelling needle is scanned laterally across the surface with a piezoelectric ceramic, the current is recorded and translated into an image, such as those in Figs 2 and 4. Our STM is operated using fast scanning rates¹¹, 1 kHz in *x*, 5–10 Hz in *y*, giving a recorded frame every 0.1 s. A feedback loop is used to keep the tunnelling current approximately constant by changing the distance between tip and surface. The bandwidth of the feedback is slightly less than the scanning rate. The needle (tip) is made of tungsten, and is electrochemically etched. The measurements described here were made in air.

A typical experiment is carried out as follows. Single-crystal (highly oriented pyrolytic) graphite provides the conductive surface. After cleaving the graphite, the surface is covered with a drop of, for example, di(2-ethylhexyl) phthalate (Fig. 1b), one of many organic species that we have found to be amenable to this experiment. The tip is then immersed in the drop and mechanically guided toward the surface. A low bias voltage is used (typically 30 mV) to image the graphite background^{12,13}. The liquid molecules act as an insulating barrier and only the graphite is seen with the STM.

The images of the surface are altered, however, when the bias voltage is raised to several volts. Figure 2a shows the graphite

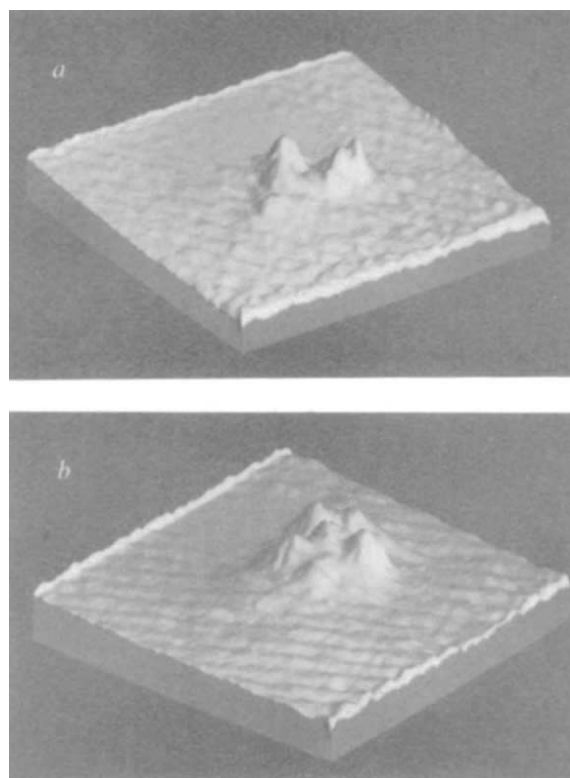


Fig. 2 *a*, STM image of a di(2-ethylhexyl)phthalate molecule pinned to the surface of graphite by a 3.7-V pulse to the tunnelling tip. *b*, A molecule pinned ~ 500 Å away by another voltage pulse at a later time. Each frame is 27×27 Å, and is taken with the tip and graphite surface surrounded by liquid di-2-ethylhexyl phthalate. The images are obtained by recording the tunnelling current as the tip is scanned laterally over the surface, while a feedback circuit maintains a constant tip-surface spacing with a bandwidth just less than the scanning frequency. The voltage bias of the tip is +30 mV and the tunnelling current is ~ 0.4 nA. A direct calibration of the heights of the bumps in the image is not possible in this case, although we estimate a maximum height differential of several ångströms. The three-dimensional view is obtained by assigning from the data a vertical height as well as a brightness on each line scan; the image is then calculated assuming a particular light-source location and diffuse reflection from the object. The shadowing effect on one side of the pinned molecule is an electronic artefact related to the way in which the data are acquired.

and adsorbate (di(2-ethylhexyl)phthalate) after a 100-ns, 3.7-V pulse has been applied to the tip. The pulse is applied during the normal scanning, the structure in the centre of the image appears immediately following the pulse. Figure 2b shows another image generated in the same manner. While the size and shape of the structures may vary, we believe that the result of the voltage pulse is the bonding of a single molecule or fraction of a molecule to the surface.

Although it is difficult to identify the di(2-ethylhexyl)phthalate molecular structure from Fig. 2, the scale of the features on the graphite surface is consistent with the molecular dimension of that species. Alternatively, the structures pinned to the graphite surface in Fig. 2 could reflect an atom or small group of atoms on the graphite surface, imaging the imperfectly shaped tip. [A sharp protrusion from the graphite surface could image the blunt tunnelling tip. Although this effect is always difficult to rule out with the STM, we note that these images were recorded within a few minutes of each other and although the detail is similar, it is not identical, nor is the orientation. If the tip shape were being imaged this effect would show up repetitively over the surface—assuming the shape was unchanging; this was not seen during these experiments.]

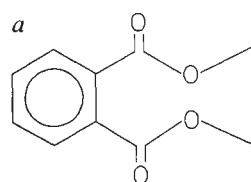
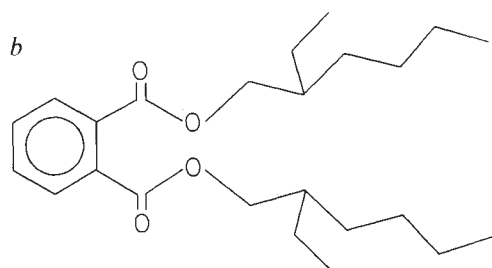


Fig. 1 Stick models of dimethyl phthalate (*a*) and di-2-ethylhexyl phthalate (*b*).



There is a voltage threshold of ~ 3.5 V for generating pinned structures, as shown in Fig. 3. We have not observed a polarity dependence in the voltage threshold. This threshold is independent of the tunnelling current used while imaging the surface before application of the voltage pulse. These tunnelling currents are typically between 0.1 and 1.0 nA. While imaging pinned structures, we do not observe any changes in the features as a function of tunnelling current and hence do not observe the anomalous distance dependence seen by Bryant *et al.*¹⁴. In addition, we have not observed a dependence of the ability to image the pinned structures on the polarity of the low-voltage bias.

The pinned molecule can be removed from the surface with another voltage pulse. The pulse is applied as the tip passes over the mark; the molecule then disappears, leaving the graphite substrate with no visible defect. As shown in Fig. 3, the 'erasing' voltage also has a threshold, which is roughly equivalent to the 'writing' threshold. Expulsion of the entire molecule from the surface is one of several observed reactions to the applied pulse; we have also observed partial erasure (see below) and enlargement. 'Enlargement' describes an increase in the spot size, presumably due to the addition of more molecules to the existing one attached to the surface.

Figure 4 shows the result of partial erasure of a pinned dimethyl phthalate molecule (Fig. 1a). Figure 4a shows a pinned molecule; Fig. 4b was obtained after a 3.7-V pulse was applied to a molecule similar to that in Fig. 4a. The pinned feature is now reduced in size, perhaps because part of the molecule has been ripped away, leaving a small portion behind. The remaining segment is the size and shape of a benzene ring, which is a component of dimethyl phthalate (see Fig. 1a). A possible explanation for this reduction in spot size is the cleavage of the two substituents from the benzene ring, constituting a chemical transformation on a single molecule.

Figure 4b also demonstrates what we believe to be the smallest spatially localized change of matter achieved in a laboratory. We have reproduced this result several times. The mark is ~ 4 Å across, which is about half the size of the mark made on germanium in ref. 4. The procedure for 'writing' on germanium is proposed to occur by shearing of occluded germanium atoms from a tungsten tip in large electric fields (in ultra-high vacuum). Our experiment uses molecules in the liquid state (a semi-infinite reservoir), and to date has been successfully performed only on

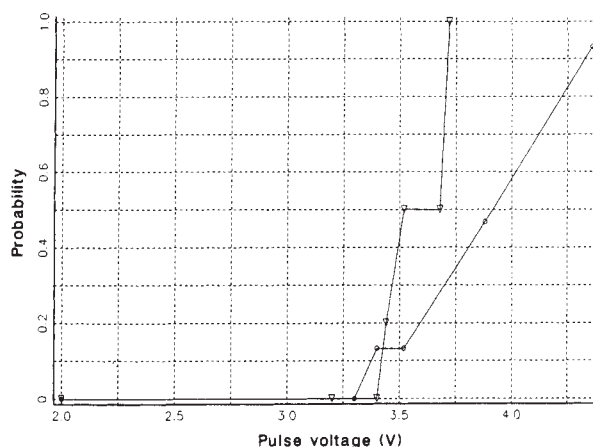


Fig. 3 Probability of pinning or removing a molecule on the surface of graphite as a function of pulse voltage applied to the tunnelling tip. The 'write' probability (○) is measured by counting the number of successful writes (independent of the size of the written feature) out of 30 attempts, at each voltage. The erasing probability (▽) is measured with 20 attempts at each voltage, all on existing pinned molecules. Partial erasures are considered successful attempts.

graphite. We have no reason to believe that the features shown in Figs 2 and 4 are derived from the tip, because behaviour indicative of tip shape change has not been observed.

We believe that 'writing' occurs when the tunnelling electrons have enough energy to activate an adsorbed molecule and/or the graphite surface, resulting in attractive forces between the two. That the threshold electron energy, ~ 3.5 eV, coincides with the energy of a typical single carbon-carbon bond could imply that covalent-bond cleavage is part of the pinning process. Alternatively, the bonding could be occurring through dipole-dipole interactions provoked by the tunnelling electrons. The evidence of partial erasure suggests that organic molecules can be cleaved by tunnelling electrons. Clearly, more theoretical and experimental work is necessary to determine the mechanics of the process. Other investigators have shown that excited molecules (H_2 , O_2) can quench unoccupied electron levels of

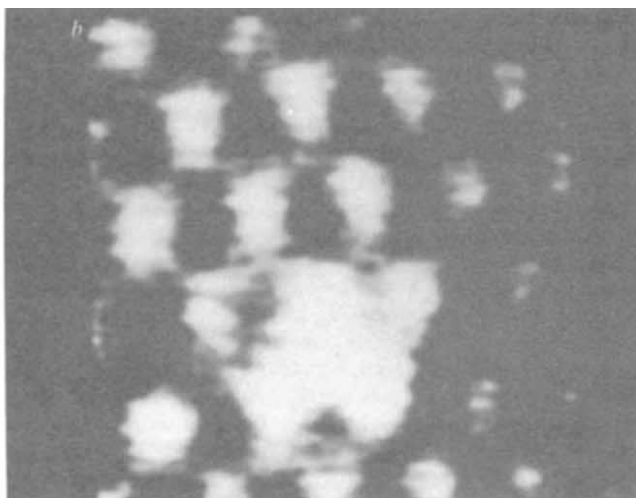
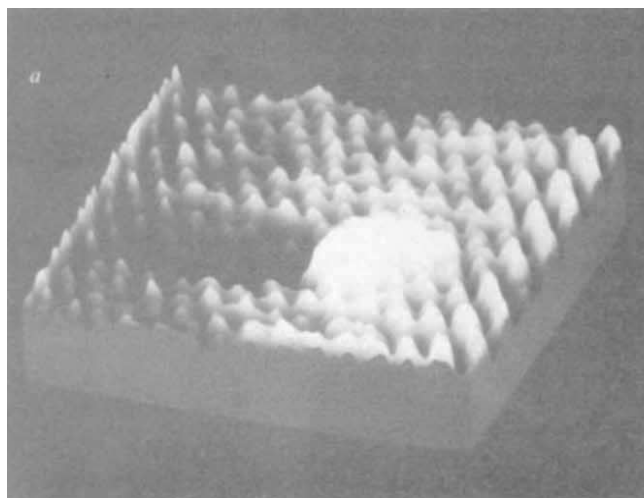


Fig. 4 *a*, Three-dimensional view of a pinned molecule with the tip and graphite surrounded by dimethyl phthalate. The frame is 34×32 Å. *b*, Two-dimensional view of the small mark remaining after the application of a 3.7-V pulse to a pinned molecule like that in *a*. The frame is 11×11 Å. The size (~ 4 Å) and shape of the feature matches a benzene ring. The mark is centred between three beta sites of the graphite surface (assuming a single tunnelling tip¹⁶), which could be either an alpha site or a hollow (H) site. The beta sites do not have an atom directly below, in the second layer of the graphite lattice. The alpha sites do have an atom directly below, and are not normally imaged with the STM. The hollow sites have an atom in the second layer but not in the first. As in Fig. 2, the shadowing effect on one side of the pinned molecule is an electronic artefact.

graphite¹⁵. If the quenching of these levels is associated with bonding of H₂ and O₂ to the surface, then an analogous mechanism for pinning the phthalate molecules to graphite could be proposed in which the voltage pulse serves to excite the phthalates. This excitation could take the form of charge transfer or bond breaking.

In an additional experiment, it appears that electron waves in graphite are scattered from a pinned molecule (H. A. Mizes and J. S. F., in preparation). In this context, the molecules can be regarded as defects in a crystal. By imaging the direct results of electron scattering from a discrete defect, information might be obtained concerning the nature (for example, strength and symmetry) of the bonding of the molecules to the surface.

Our interpretation of this process as the pinning, removal and cleaving of the phthalate molecules is still open to question. The STM images themselves show with certainty that we are able to reversibly mark a surface on a molecular scale, and make smaller marks from larger marks, by applying pulses over a critical voltage threshold. It is possible that the mark is a small group of atoms transferred from tip to surface or surface to tip⁴. The transfer could be thermally mediated, or caused by the momentum of the tunnelling electrons. Additionally, 'defects' induced in a graphite surface can produce surface reconstruction (H. A. Mizes and J. S. F., in preparation). While these alternative explanations are possible, tunnelling-electron-induced bonding is an attractive and simple picture to explain our observed reversible results.

Although much work remains to be done, we believe that

tunnelling microscopy is on the threshold of a revolution in manipulating atoms and molecules for a variety of purposes. We have demonstrated a viable technique for pinning discrete molecules to the surface of graphite, and have shown that these molecules can be further manipulated and possibly chemically altered. These techniques show promise for technological applications, in that the molecules are derived from an infinite reservoir and altered with sub-microsecond speed.

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Synthesis, structure and superconducting properties of metastable

Y(Ba_{2-x}Y_x)Cu₃O_{7+δ}

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The oxygen-deficient orthorhombic oxide YBa₂Cu₃O_{7-δ}¹⁻³ has been the centre of intense recent interest because of its bulk superconducting properties near 90 K. More recently the closely related oxide, La_{1.5}Ba_{1.5}Cu₃O_{7+δ}, is receiving increased attention⁴⁻⁷. The yttrium analogue, Y_{1.5}Ba_{1.5}Cu₃O_{7+δ}, has not been successfully synthesized by the usual ceramic routes involving firing at temperatures above 900 °C^{8,9}, because of its intrinsic metastability. Here we report on the synthesis and characterization of a majority (90-95%) phase Y_{1.5}Ba_{1.5}Cu₃O_{7+δ} (referred to for historical reasons⁸ as Y 3-3-6), prepared by firing an atomically mixed citrate precursor between 810° and 825 °C for 2 h. X-ray powder diffraction data indicate that Y 3-3-6 is isostructural with its tetragonal La analogue^{4,7}. Electron microprobe and thermogravimetry data yield a unit-cell composition Y(Ba_{2-x}Y_x)Cu₃O_{7+δ}, with $x=0.50$ and $\delta=0.10-0.15$ for the parent compound. Specimens of Y 3-3-6 with $x=0.50$ and 0.375, which were annealed in O₂ at 1 atm and >3 atm, displayed semiconducting behaviour. Filamentary superconductivity, detected electrically and magnetically, with transition onsets at 80 and 60 K respectively, is observed in specimens annealed in 3 atm O₂ at 520 °C.

Specimens of Y 3-3-6 with $x=0.50$ and 0.375 were synthesized from a precursor consisting of the atomically mixed citrates of

Y, Ba and Cu. The precursor is prepared by individually dissolving copper and barium acetate and yttrium nitrate in water at ~30 °C. The solutions are then mixed and an aqueous solution of citric acid added to quantitatively convert the copper and barium acetates and the yttrium nitrate to the corresponding citrates. With the pH held at ~2-3, the solution is evaporated to a blue gel. The gel is then transferred to a boat and calcined in air at 500 °C for 30 min to 1 h, after 15 min at 150 °C to remove the last traces of water. It is important to avoid calcining for more than 1 h because crystallization of the oxides results in decreased atomic mixing. The calcined powder is then ground under acetone, dried and fired as a powder in a tube furnace between 810 and 825 °C for 2 h under flowing oxygen. The temperature of the furnace is then lowered slowly at 30 °C. The powdered material is then pelletized and annealed at 650 °C for 9 h under flowing O₂, or at 520 °C under various pressures of O₂ above 1 atm. The stable La 3-3-6 and La_{0.75}Y_{0.75}Ba_{1.5}Cu₃O_{7+δ} (La, Y 3-3-6) compounds were prepared by the usual ceramic route of firing the metal oxides and BaCO₃ at 950 °C for 24 h, as described in refs 7 and 8.

Figure 1 shows the X-ray diffraction patterns of a Y 3-3-6, $x=0.50$ sample and of the stable La 3-3-6 parent phase, together with a calculated diffraction pattern based on the La 3-3-6 coordinates reported in refs 4 and 7 and the lattice parameters extracted from the Y 3-3-6 data. The X-ray reflections of the majority phase (>90-95%) in the Y 3-3-6 sample correspond to the lines in the diffraction pattern of the La 3-3-6 material and the calculated pattern. The reflections can be indexed to a tetragonal phase which is a variant of YBa₂Cu₃O_{7-δ}¹⁰. The lattice parameters obtained from the data are $a=3.876$ Å, $c=11.628$ Å. Note that the c -axis parameter for Y 3-3-6 is appreciably lower than that reported for La 3-3-6^{4,7} and YBa₂Cu₃O_{7-δ}². The weak (100) reflection reported by Er-Rakho *et al.*⁸ at a d -spacing of ~5.5 Å was not observed in our Y and La 3-3-6 data. Apart from a few weak features due to some Y₂O₃ in this specimen, all of the weak lines indicated by asterisks in the diffraction pattern of Y 3-3-6 in Fig. 1 can be assigned to the semiconducting Y₂Cu₂O₅ phase. The Y₂Cu₂O₅ features are indicated by asterisks in Fig. 1.

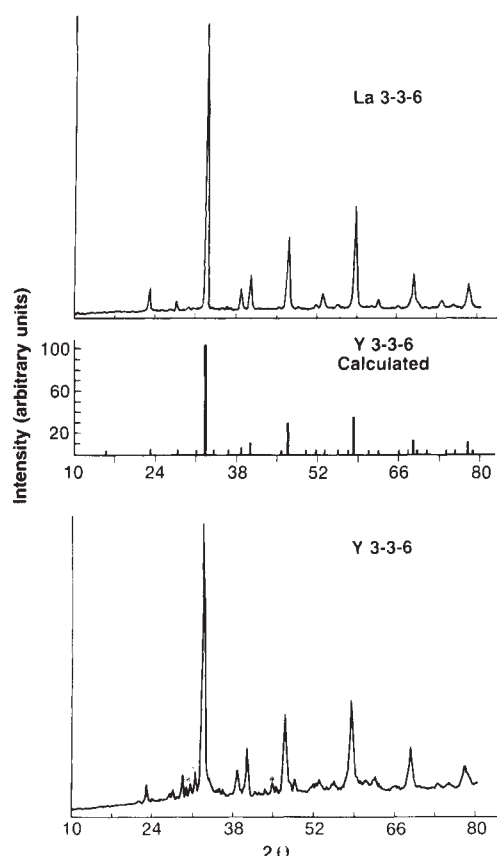
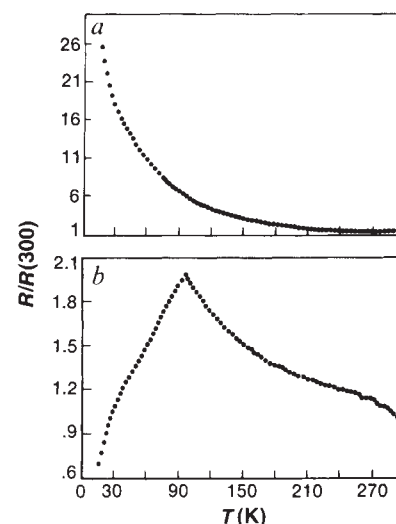


Fig. 1 X-ray powder diffraction patterns (Cu $K\alpha$ radiation) between 2θ values of 10 and 80° for La 3-3-6 and Y 3-3-6. The lines marked with an asterisk are associated with $Y_2Cu_2O_5$. Also shown is a calculated line pattern for Y 3-3-6 (see text for details of the calculation).

The presence of $Y_2Cu_2O_5$ in the final product suggests that some of the barium carbonate formed from the citrate during initial calcination may not have undergone complete solid-state reaction at the relatively low temperatures and short firing times employed in the final stage. The barium carbonate in the calcined precursor can be converted to the nitrate by reaction of the precursor with HNO_3 , followed by neutralization of the excess HNO_3 with NH_4OH . By firing this converted precursor containing the more labile barium nitrate, it is possible to reduce the $Y_2Cu_2O_5$ impurity content and obtain a purer (as determined by X-ray diffraction) Y 3-3-6 phase.

The electron microprobe results for Y, Ba and Cu on representative Y 3-3-6 samples (referenced against a standard $YBa_2Cu_3O_{7-\delta}$ (Y 1-2-3) sample prepared by the citrate route), which were averaged over a spot size of $1\ \mu m$, are summarized in Table 1. Scanning electron microscopy shows a very uniform grain-size distribution, with an average grain size near $1\ \mu m$.

Fig. 2 Temperature dependence of the resistivity (normalized to that at 300 K) of a Y 3-3-6, $x=0.5$ sample: a, annealed in 1 atm O_2 at $650^\circ C$; b, annealed in 3 atm O_2 at $520^\circ C$.



The observed atomic percentages of Y, Ba and Cu are in good agreement with the values expected for the Y 3-3-6 composition (Table 1). The oxygen contents were determined by thermogravimetry in a 5% H_2 -95% Ar atmosphere. The values given in Table 1 are from thermogravimetric plateaux near $500^\circ C$, which correspond closely to values obtained from the plateau at $750^\circ C$. In the latter plateau the end-product has been shown to be composed of $YCuO_2$, BaO and Cu (P. K. Gallagher, personal communication). The oxygen results were checked against a standard Y 1-2-3 sample prepared from the citrates, whose average oxygen content had been determined independently by neutron diffraction (A. Santoro and Z. Iqbal, unpublished data). For the formula unit $Y(Ba_{2-x}Y_x)Cu_3O_{7+\delta}$, δ was found to be 0.11 for the pure Y compound and 0.15 for the Y:La compound at $x=0.5$. With decreasing x the value of δ decreases but rises again after high-pressure O_2 annealing (see Table 1).

X-ray diffraction studies as a function of temperature using a sample hot-stage indicate that the Y 3-3-6 phase is unstable above $880^\circ C$ at 1 atm O_2 or air. Above this temperature disproportionation to orthorhombic $YBa_2Cu_3O_{7-\delta}$, Y_2BaCuO_5 and CuO occurs. The disproportionated samples show bulk superconductivity (measured both magnetically and electrically) with transition temperatures between 60 and $90\ K$, which is associated with Y 1-2-3. The metastability of Y 3-3-6 is most probably a consequence of the partial doping of the Ba layer in the Y 1-2-3 lattice with Y ions of much smaller ionic radius and greater positive charge. The excess oxygen atoms, which probably occupy the positions between the Cu-O chains of the 1-2-3 lattice, are required in the 3-3-6 structure to balance the extra positive charge in the Ba layer due to the presence of Y^{3+} ions.

Four-probe a.c. resistivity data (with typical currents ranging from 0.1 to 10 mA at 100 Hz) are shown in Fig. 2a for specimens of Y 3-3-6, $x=0.5$ annealed in 1 atm O_2 at $650^\circ C$ and then

Table 1 Electron microprobe results (averaged values) and thermogravimetrically determined oxygen contents (atoms per formula unit) in different 3-3-6 samples

Sample	O content	At. % Y	At. % Ba	At. % Cu	Observed Y:Ba	Theoretical Y:Ba	Observed Y:Cu	Theoretical Y:Cu
Y 3-3-6								
$x=0.50$ (1 atm O_2)*	7.11	24.03	25.87	50.10	0.93	1.00	0.48	0.50
$x=0.375$ (1 atm O_2)	7.04	22.67	28.74	48.59	0.79	0.85	0.47	0.46
$x=0.375$ (3 atm O_2)	7.33	—	—	—	—	—	—	—
La, Y 3-3-6† (1 atm O_2)	7.15	—	—	—	—	—	—	—
La 3-3-6† (1 atm O_2)	7.35	—	—	—	—	—	—	—

* Annealing conditions are given in parentheses.

† These samples were >99% single-phase as determined by X-ray diffraction.

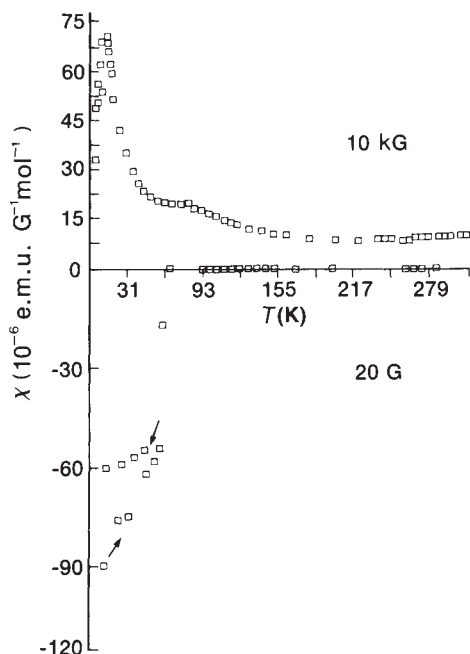


Fig. 3 Temperature dependence of the magnetic susceptibility of the same sample as in Fig. 2b, measured at 10 kG and 20 G. The arrows pointing to increasing and decreasing temperature in the lower diagram represent the exclusion and expulsion (Meissner) experiments, respectively.

slowly cooled. The observed localization below ~ 100 K is also shown by a similarly prepared Y 3-3-6 at $x = 0.375$. After annealing the same samples in 3 atm O_2 at $520^\circ C$, both specimens show a superconductive transition onset near 80 K. Figure 2b shows the data for Y 3-3-6, $x = 0.5$, in which a resistive transition onset near 80 K and a shoulder at 60 K can be seen. Figure 3 shows d.c. susceptibility data for the same high-pressure-annealed sample, measured with a SQUID magnetometer at magnetic fields of 10 kG and 20 G. In low field, both exclusion and expulsion experiments indicate the onset of superconductivity below 60 K in a small (1–5%) volume fraction of the sample. This result is similar to that observed in some samples of La_2CuO_{4-x} ^{11,12}. The high-field data suggest strong localization below 30 K, with a singularity at 60 K corresponding to the onset of the diamagnetic signal in the low-field data. The same sample, when annealed in 10 atm O_2 , shows no indication of a superconducting fraction. These results suggest that when the oxygen content is greater than seven oxygens per formula unit in the Y 3-3-6 structure, the crucial Cu–O chains of the Y 1-2-3 lattice are disrupted, resulting in a loss of bulk superconductivity in the material. Some chain ordering in a minority component of the sample may result in the filamentary superconductivity observed on annealing near an optimal O_2 pressure of 3 atm.

The formation of quasi-icosahedral spiral shell carbon particles

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A simple, new, carbon nucleation scheme has been developed which results in quasi-single crystal particles of concentric, spiral-shell internal structure and overall quasi-icosahedral shape. Intermediates consisting of curved graphitic networks and overall growth controlled by epitaxy are key factors in the scheme which also produces buckminsterfullerene, C_{60}^B , as an inert by-product. The scheme which explains, for the first time, the occurrence of polyhedral carbonaceous particles may also apply to soot and circumstellar dust formation.

Until recently it has been assumed that carbonaceous particles such as soot form by high-temperature carbonization of liquid hydrocarbon droplets, the shapes of which are governed by surface tension. This view has been modified by transmission electron microscopy (TEM)^{1–3} showing the particles as regular concentric graphitic shells. Similar structures have been found in condensed carbon⁴ for which a liquid step seems improbable. This similarity suggests the formation mechanisms are related. Tesner⁵ argues that some form of ‘chemical crystallization’ must be invoked to explain the regular structure of soot. The discovery of the superstable C_{60} molecule with properties consistent with a closed, truncated, icosahedral sphere⁶ has led to a new description of a family of hollow-cage carbon species (fullerenes)⁷. A scheme has also been suggested⁸ for the formation of C_{60}^B and also of macroscopic carbon particles. Here we discuss a refined nucleation scheme that explains why polyhedra develop. The study further supports the proposal that C_{60}^B is indeed a closed cage⁶.

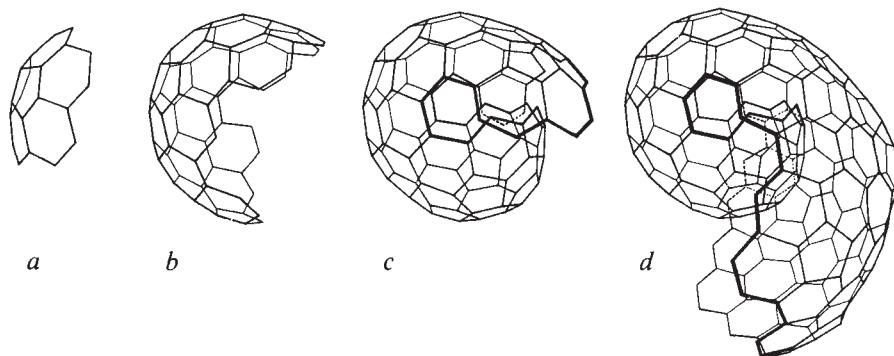
The nucleation scheme rests on the validity of the fullerene proposal and its implications. Traditionally, extended carbon aggregates are expected to involve flat, hexagonal graphitic networks, and it is proposed that the exceptional stability of C_{60}^B is consistent with the closure of such a network and the consequent elimination of the reactive edge of an open sheet. A purely hexagonal network cannot close perfectly, but if 12 pentagons are incorporated it can, although the number of hexagons is arbitrary^{9,10,11}. Chemical and geodesic arguments indicate the fullerenes should be stable^{7,12,13} and that the set with 24, 28, 32, 50, 60 and 70 atoms would be particularly stable⁷, consistent with observation. C_{60}^B is predicted to be uniquely stable because it is the first cage to avoid abutting pentagons, it is highly aromatic, and its high symmetry enables it to distribute strain perfectly in a way available to no other 5/6-ring cage.

Most important is the proposal that carbon nucleation can lead to cages spontaneously. The first key element in the process is the formation of curved 5/6-ring networks by the energy-driven urge to eliminate edge-dangling bonds. In such a chaotic aggregation process the pentagons, which are crucial for curvature, are likely to be dispersed more or less randomly, producing structures such as those in Fig. 1. Occasionally however, they will occur at just the correct place for perfect closure, producing C_{60}^B (ref. 6) or other fullerenes⁷. Without a reactive edge, fullerenes should not grow further but should remain intact in a cul-de-sac while the unclosed shells, presumably the majority, race on to form large particles. The fullerenes are unsuitable sites for further growth and so C_{60}^B will not generally be found at the centre of a carbon particle. Later closure by annealing or accidental entrapment should not, however, be ruled out. For most clusters the advancing edges will miss (Fig.

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Fig. 1 Hypothetical initial growth sequence evolving from: *a*, a corannulene carbon framework through *b*, and *c*, species in which edge by-pass occurs to *d*, an embryo in which the second shell is forming. The sequence leads in a natural way to both carbon particles and closed cages, fullerenes.



1c), and once overlapped, closure is impossible and further rapid growth is predicted to be like a snowball accreting a more or less continuous spiral layer (Fig. 1d). Accreting species, on collision with the energy-absorbent surface of the embryo, should be adsorbed and rapidly assimilated into the advancing skin.

The model needs to be refined to deduce structural details. (1) The curvature of the initial embryo should be governed by the 5/6-ring energetics of a free sheet under the condensation conditions that obtain and, although fairly round at first, rather abruptly angled facets may develop, particularly when edge overlap occurs. (2) As accreting species become bonded into the advancing network, pentagons should be located at strategic points so that fresh surface can follow the underlying convex contours, optimized for the graphite interlayer separation (3.4 Å). An important consequence is that new pentagons in the fresh layer should form directly above those in the layer immediately below, so propagating structural integrity. Thus the pentagons should stack epitaxially in columns along twelve radii. (3) As the spirals grow and their sizes become large relative to the interlayer spacing, the overall shapes should approximate to those of closed cages of similar dimensions. Interlayer adhesion, which may be important, is neglected in this preliminary study. (4) Various types of discontinuity should occur. For instance two helical edges propagate as the spiral grows. In Fig. 1 the skew axes are opposed, so the edges spiral away from each other. Normally random discontinuities in the network probably also occur, spoiling the quality of the surface.

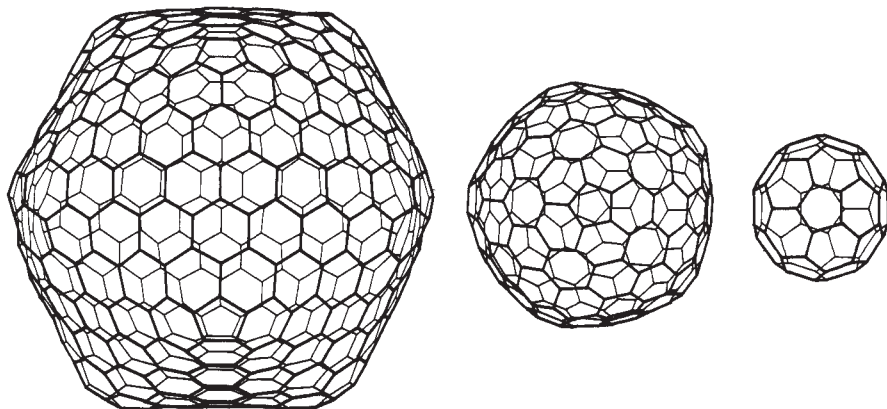
The hypothetical sequence depicted in Fig. 1 is based on these guidelines, and the initial embryo is a fairly smoothly curving shell with a 5/6-ring ratio close to 12/20 (presumably an optimum under certain conditions based on the formation and stability of C_{60}^B). As the structure grows, flat segments develop, resulting in a mis-shapen, shell-like nucleus with a 7–10 Å diameter spiral (Fig. 1d), which is probably the minimum size for a suitable growth core. Larger initial structures may occur and their preponderance could depend on nucleation conditions.

To predict the likely shapes of the resulting particles, various large cages have been constructed. Schematic diagrams of C_{60} , C_{240} and C_{540} (of diameters $1D$, $2D$ and $3D$, where D is that of C_{60}), related to the Goldberg polyhedra^{13–15}, are depicted in Fig. 2. The main structural features of $C_{1,500}$ and $C_{6,000}$ are to be found in C_{540} . These cages show a major structural feature as the cages grow, the round shape disappears and flat faces develop rapidly. Even though there are only 12 pentagons in any given shell (the number of hexagons is proportional to D^2), they exert a profound influence on the shapes of the large shells. The corannulene configuration introduces a rigid cusp in the surface, the shape of which is determined by the carbon-carbon aromatic bond lengths¹⁶. The graphitic sheet linking the 12 pentagons follows the tangential angles which radiate out from the saucer-shaped cusps, and warps so as to maintain as planar an interlinking surface as possible. The resulting shape is quasi-icosahedral in that it consists of 20 more-or-less flat, but smoothly interconnected, triangular planes. The cross-section of a large symmetric cage is a 6–10 sided polygon, depending on the cut, and in a random-growth scheme, unsymmetric polyhedra with $\sim 8 \pm 2$ -sided polygonal cross-sections should occur.

For a large particle consisting of a spiral surface based on concentric polyhedral segments, epitaxial growth causes the triangular planes in successive shells to lie directly above one another, and the interconnecting folds to lie in triangular planes radiating from the nucleus. The model thus predicts a quasi single-crystal particle composed of 20 pyramidal microcrystallite segments. The flat layers out of which any one microcrystallite is formed fold smoothly into the layers of the three abutting microcrystallites, and are parallel to the outer surface. Due to epitaxy, the cross-section should exhibit a spiral pattern of concentric, aligned polygons, albeit with defects.

These predictions can be tested experimentally. In 1980 Iijima⁴ published TEM pictures of spheroidal carbon particles. A highly spherical example is shown in Fig. 3a, and a less regular and more clearly polyhedral one in Fig. 3c. A planar array four or five atoms wide, within a 2 Å-thick wall, parallel to the TEM beam, manifests itself as a dark line⁴. This condition can be met

Fig. 2 A set of models for the closed symmetric fullerenes C_{60} , C_{240} and C_{540} . The diameters of the latter cages are 2 and 3 times that of C_{60} . The rapid shift towards quasi-icosahedral symmetry is striking.



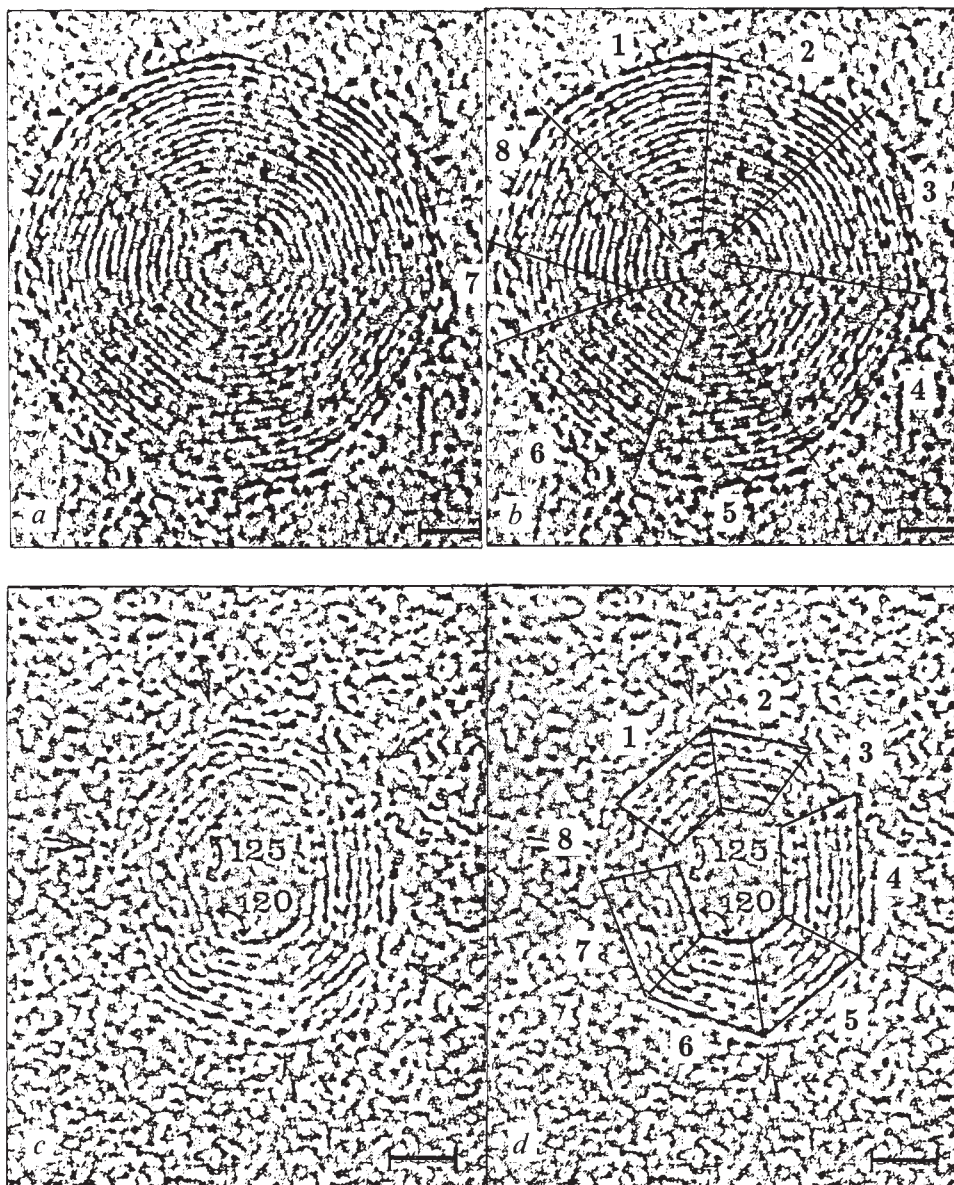


Fig. 3 *a, c*, Images of two published micrographs. On the right (*b, d*) these images are marked to delineate the features consistent with pyramidal layered microcrystallite cross-sections, as predicted. 20 Å markers are appended.

by almost any contour of a cage. Iijima has recently discussed a small central structure in one of his particles¹⁷ which we interpret as an embryo of the kind shown in Fig. 1*d*.

The TEM patterns in Fig. 3 can now be readily explained. In the region of the nucleus in Fig. 3*a* there are discontinuities as well as curved and straight contours, consistent with an embryo such as Fig. 1*d*. Triangular segments that are consistent with cross-sections through pyramidal microcrystallites are delineated in Fig. 3*b*. Smooth-layer inter-connections are clearly visible at the 2/3 boundary and less so at the 8/1, 1/2 and 4/5 boundaries. Discontinuities, as expected, are seen in several regions, such as the 3/4, 5/6, 6/7 and 7/8 boundaries. Segment 7 appears to originate from a secondary growth point some 30 Å out from the centre, indicating the possibility of secondary and multiple growth-sites. Discontinuities near the cusps could result from incomplete formation of corannulene configurations by avoidance of pentagon formation. The angles expected should vary roughly between those for the isocahedral dihedral angle 138.2° (ref. 18), and that for corannulene of 122.8° (ref. 16). All the Iijima micrographs are consistent with polyhedral structures. Here we see that the closure criterion^{9,10,11} is satisfied most simply on a microscopic scale at the cusps using only sp^2 bonding.

The particle in Fig. 3*c* is clearly polyhedral with distinct layer patterns in segments 1, 2, 4, 5, 6 and possibly 7, and discontinuities in 3, 7 and 8. The central spiral is 30–40 Å across, significantly larger than that in Fig. 3*a* and, as predicted, more distinctly polyhedral. The smaller the embryo, the more spheroidal the ultimate particle may be. Conglomerates resulting from multiple growth sites are explained by a straightforward extension of the model.

The close relationship between carbon chains and particles in circumstellar shells^{19–21} led to the discovery of C_{60}^B (refs 6, 22) and ultimately to the present polyhedral structure proposal. Circumstellar dust is ejected into space, and so it is possible that such polyhedral particles are the primary refractory cores which are the accretion sites linking the gaseous component of the interstellar medium with large objects such as planets and stars. Also, it is likely that the symbiotic triangular relationship observed in the laboratory between polyyne chains, polyhedral shell particles and C_{60}^B , also exists in the interstellar medium. As C_{60}^B can apparently withstand destruction better than any other chemically bound species^{21–25}, it is likely to be distributed as ubiquitously in the Galaxy (probably ionized) as dust and chains^{26,27}. Admixed hydrogen will perturb the spiral structures, although not seriously if refractory carbonaceous particles occur

at all. Hydrogenated polyhedral shells are essentially three-dimensional polyaromatic hydrocarbons (PAH) and should show PAH-type infrared emission²⁸. Thus, partially hydrogenated particles are possible carriers of the Unidentified Infrared Emission Features which have features in common with those of PAH material²⁹.

The relationship between chains, grains and C_{60}^B in space seems to be paralleled in that of acetylenes with soot¹⁹. This possibility is supported by the discovery of C_{60}^+ in a sooting flame³⁰. Hydrogen complicates matters, probably making oxidative coupling more important in the polymerisation reaction step than free radical condensation, and perhaps obscuring some of the structural details of the resulting particles. It is unlikely that the overall mechanics of the soot-formation process differ much from that proposed here, because Iijima's particles and soot have so much in common, and two parallel processes to form such similar and unusual structures would be unlikely. The mechanism is readily modified for hydrocarbon combustion by realizing that soot should form when there is oxygen starvation, and the temperature is such that the large C—C bond energy, relative to C—H and C—O, ensures that hydrogen and oxygen insertion cannot compete with carbon-network formation. The study thus implies that soot should also show a quasi-icosahedral spiral layer structure, albeit modified by H and O inclusion, a proposal supported by the plethora of 5-membered ring compounds found in tars³¹. As for kinetic factors, it is likely that the surface accretion step is rate-limiting, as polyene cross-linking is very fast, indeed explosive, and so the model is consistent with a growth rate proportional to the surface area of the particle.

Finally, the structures considered here rather curiously possess both icosahedral and helical features. If they are soot-like, they must have been abundant in the Earth's early atmosphere, providing useful prebiotic nucleation sites.

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Formation of highly orientated graphite from polyacrylonitrile by using a two-dimensional space between montmorillonite lamellae

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Carbon materials have many attractive physical and chemical properties, because of a wide variety of chemical bonding, crystal structure and microtexture. Carbon properties can be created by selecting a preparation procedure and a raw material. For example, many new carbon materials such as exfoliated graphite, benzene-derived fibre and diamond film have come into use recently. The possibility of creating a new carbon material by a new method still remains. Here we report a novel method of preparing a highly orientated graphite from polyacrylonitrile (PAN) by making use of the interlamellar opening of montmorillonite (MONT) as a two-dimensional space for carbonization. A MONT-PAN intercalation compound was prepared and heat-treated at 700 °C to produce carbon from PAN between MONT lamellae. The carbon was then released from MONT by acid treatment and subjected to further heat treatment at various temperatures up to 2,800 °C. The interplanar spacing (d_{002}) of the carbon treated at 2,800 °C was very close to that of ideal graphite crystal and the crystallite size, L_c and L_a , were ~40 nm and >1 µm, respectively. The formation of such a highly orientated carbon is probably a consequence of the orientation of the two-dimensional carbon precursor produced between the lamellae of MONT. When PAN itself is heat-treated, it produces a three-dimensional carbon with a network of intertwined ribbons of stacked graphitic sheets.

MONT is a typical layered clay mineral which forms intercalation compounds with various kinds of polar organic molecules. We attempted to use a two-dimensional opening between the lamellae of MONT as a carbon-producing centre intercalated polymer compounds, expecting to obtain a low-dimensional carbon which may have interesting properties^{1–3}.

For this purpose, we first prepared MONT(Ca form)-acrylonitrile complex^{4,5}, using 0.14 g of intercalated monomer per gram of MONT. The complex was subjected to γ-ray radiation at room temperature under nitrogen to polymerize acrylonitrile between the lamellae. The formation of PAN was confirmed by Fourier transform infrared measurements. The MONT-PAN complex was then heat-treated under flowing nitrogen. *In situ* X-ray diffraction measurements revealed that the interlamellar spacing of the complex was ~1.3 nm below 300 °C, a spacing which corresponds to the presence of monolayer PAN. The polymer decomposed between 400–550 °C and formed flat carbonaceous molecules, and this change resulted in a slight

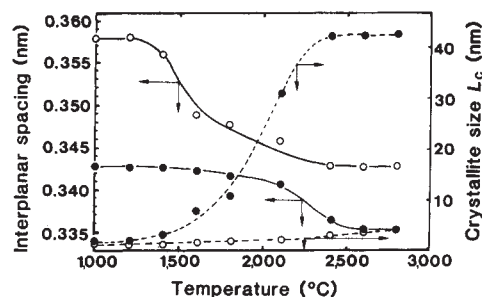


Fig. 1 Changes of the interplanar spacing d_{002} and the crystallite size, L_c , of IPC (●) and FPC (○) as a function of HTT.

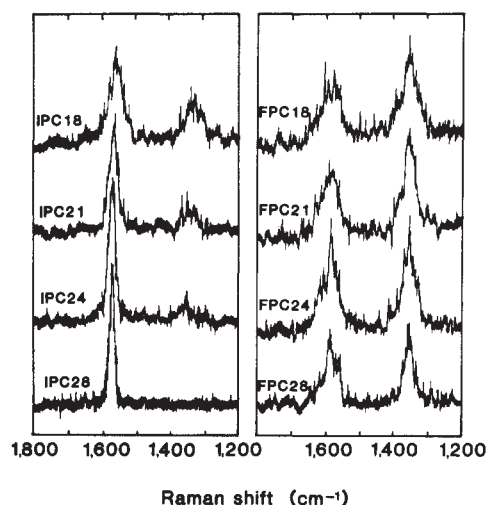


Fig. 2 Raman spectra of IPC and FPC heat-treated at different temperatures.

decrease of spacing to ~ 1.1 nm above 600°C . The carbon heat-treated at 700°C for 3 h was liberated from MONT by successive washing with HF and HCl solutions. Elemental analysis indicated that the carbon content of the intercalated polymer-derived carbon (IPC) was 65 wt%, considerable amounts of nitrogen and oxygen being observed. IPC was subjected to further heat treatment at various temperatures up to $2,800^\circ\text{C}$. For comparison, a carbon from ordinary PAN (free polymer-derived carbon, or FPC) was prepared as a reference. The number following the term IPC or FPC in this paper indicates one hundredth of the heat-treatment temperature (HTT) in degrees Celsius.

Elemental analyses of IPC and FPC at various HTTs indicate that the elimination of hetero-atoms was almost completed at just below $1,600^\circ\text{C}$. Scanning electron microscopy of FPC and IPC shows clear differences in structure: FPC has a block-like structure, whereas IPC seems to consist of many thin films. The apparent shapes of these carbons were almost independent of HTT.

Figure 1 illustrates the interplanar spacing, d_{002} , of IPC and FPC as a function of HTT. The interplanar spacing of FPC at $1,000^\circ\text{C}$ (0.358 nm) rapidly decreased to 0.349 nm at $1,600^\circ\text{C}$, and then gradually decreased to 0.343 nm at $2,800^\circ\text{C}$. For IPC, however, spacing was as low as 0.343 nm at an HTT of $1,000^\circ\text{C}$, falling to 0.3355 nm following heat treatment at $2,800^\circ\text{C}$ (this value was calculated from 004 reflection). The crystallite size, L_c , was calculated from the peak width of X-ray 002 diffraction using Scherrer's equation. The difference in L_c between the two forms of carbon is also remarkable: L_c for FPC increased only marginally on heat treatment, whereas that of IPC rapidly increased, reaching 42 nm at a HTT of $2,400^\circ\text{C}$ and above.

Figure 2 shows Raman spectra for both carbons. Scattering bands at $1,580$ and $1,360\text{ cm}^{-1}$ are considered to be due to graphite-like structure and disordered-structure of carbon, respectively. Comparison of these two series of spectra clearly indicates that IPC has a much greater potential for graphite formation than does FPC. The absence of a $1,360\text{ cm}^{-1}$ band is taken to be good evidence for perfect crystallization.

The bright-field micrograph of IPC28 shows this sample to be composed of thin films which are almost transparent against the electron beam. Dark bundle-like images 5 – 50 nm thick and 0.1 – $5\text{ }\mu\text{m}$ long are also observed. The dark-field micrograph indicated that this bundle consists of 002 planes which are parallel to the electron beam. It was presumed that IPC28 film is made of wrinkled graphitic sheets and the dark bundle-like images correspond to vertical parts of wrinkled sheets. Figure 3a, b shows the 002 lattice image and selected area electron

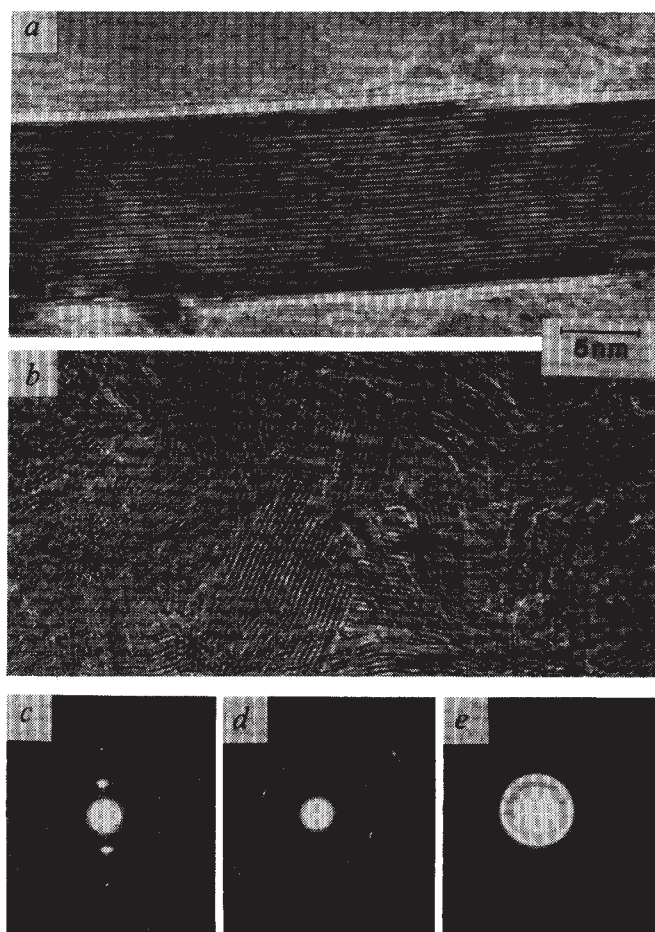


Fig. 3 002 Lattice image for IPC28 (a) and FPC28 (b), and the SAD patterns for a vertical part of IPC28 (c), a flat part of IPC28 (d) and for FPC28 (e).

diffraction (SAD) patterns for a vertical part of IPC28 film. The image shows that this part consists of regularly stacked graphite layers, and there are some 40 layers in this case. The crystallite size, L_a , can be estimated from moiré-image interference fringes and is $>1\text{ }\mu\text{m}$. This large L_a is one of the most remarkable features of this new form of carbon. The SAD pattern obtained for this vertical part presents pairs of relatively sharp spots for 002, 004, 006, 008 diffractions and weak rings for 100, 110, 200 diffractions. In contrast, the SAD in the area of the flat part of film (Fig. 3d) revealed a chain of sharp spots for $hk0$ diffractions but no 00l peaks. The film thus consists of several perfect crystallites which are highly orientated parallel to the film plane.

A sample of carbon formed from ordinary PAN, however, consists of a network of intertwined ribbons of stacked graphitic sheets with 8–15 layers (FPC28 in Fig. 3b). This structure is similar to those observed for so-called glass-like carbons⁶. In the SAD pattern (Fig. 3e), 002 and very weak 10, 004, 11 diffractions appear as continuous rings. This means that the orientation of basal planes in FPC is very poor, that is, FPC is a non-graphitizing carbon. The graphitization might be hampered by the cross-linked network structure of precursor.

Our results demonstrate that a highly-orientated graphite film can be produced by heat-treating IPC prepared between the lamellae of MONT, and that an almost ideal graphite structure was attained at as low a temperature as $2,800^\circ\text{C}$. The degree of graphitization is comparable with highly orientated pyrolytic graphite prepared under much more severe conditions ($3,500^\circ\text{C}$, 25 MPa).

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Gravity domains and assembly of the North American continent by collisional tectonics

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Trends of geophysical anomalies have made important contributions to local and regional structural studies within the North American continent^{1,2}, particularly in examining the Precambrian basement underlying the relatively undeformed Phanerozoic sediments that cover ~45% of North America³⁻⁵. Here we present a gravity trend map of North America, based on a horizontal Bouguer gravity gradient map⁶ produced from gravity data for Canada and the conterminous United States, and use it to define a continental mosaic of gravity trend domains akin to structural domains. Contrasting trend characteristics at gravity domain boundaries support the concept of the outward growth of the continent primarily by accretionary (collisional) tectonics. Gravity patterns, however, indicate a different style of tectonics dominated in the development of now-buried Proterozoic orogenic belts in the south-central United States, supporting a view that these belts formed along the leading edge of a southward-migrating Proterozoic continental margin⁷.

Recently, the usefulness of horizontal gravity gradients in enhancing Bouguer gravity anomaly trends has been demonstrated using a North American data set^{5,6}. The data and details of the derivative procedures have been illustrated and described previously^{6,8}. Horizontal gradient maps effectively highlight flanks of anomalies marking abrupt lateral changes in, generally, upper crustal densities, while at the same time suppressing more regional gradient signatures of broader anomalies caused by more gradual or deeper density changes, such as those related to the compensation of large-scale topography. Suppression of longer wavelengths also aids in comparing and identifying trends and patterns associated with various structural domains.

The North American data set of the horizontal gradient of gravity is used here to define for the first time a gravity trend map covering most of the continent (Fig. 1), from which is derived a map of first-order gravity/structural domains (Fig. 2). A distinction between gradient features greater and less than $1.25 \text{ mGal km}^{-1}$ is useful in recognizing first-order structural features in exposed and buried areas of the North American craton. The continental gravity domains have been defined largely on the basis of internally consistent sets of sub-parallel horizontal gravity gradient features and the presence of discordant trends at domain boundaries.

The structural significance of gravity trends and domains is demonstrated, for example, by a close correspondence between

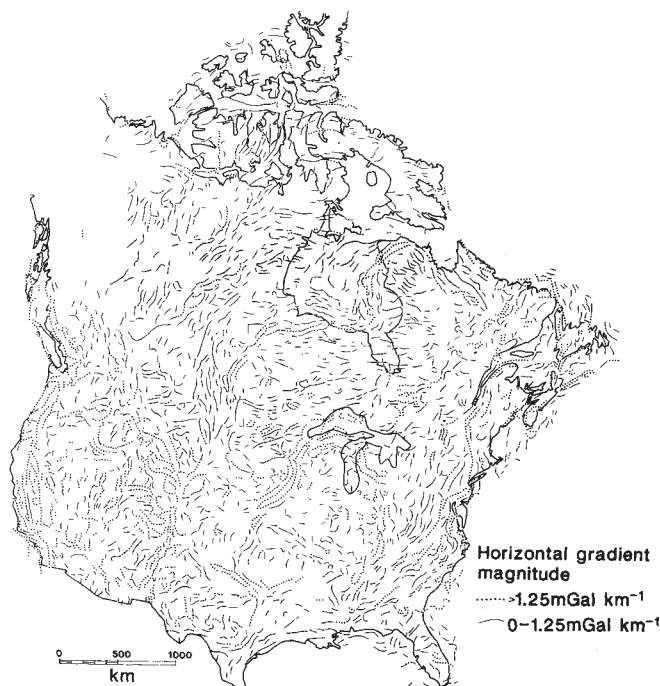


Fig. 1 Line-drawing gravity trend map of North America derived by tracing axes of horizontal gravity gradient features.



Fig. 2 First-order gravity domains of North America derived from Fig. 1 (see text). Arrows indicate growth path of continent. Successively younger terrains, believed to be accreted by collisional plate tectonics, are encountered along the arrows. Dashed arrows in Domain II indicate outward growth from the Archean Slave Province, which is not reflected as a gravity domain at this broad scale.

certain gravity domains and structural domains based largely on surface geology in the Canadian Shield. This relationship has been used to extend the exposed Superior (VIII in Fig. 2) and Grenville (XI) provinces and the Trans-Hudson orogen⁹ (VII) beneath Phanerozoic cover^{5,6}. The largest gradient magnitudes within the shield generally occur along boundaries

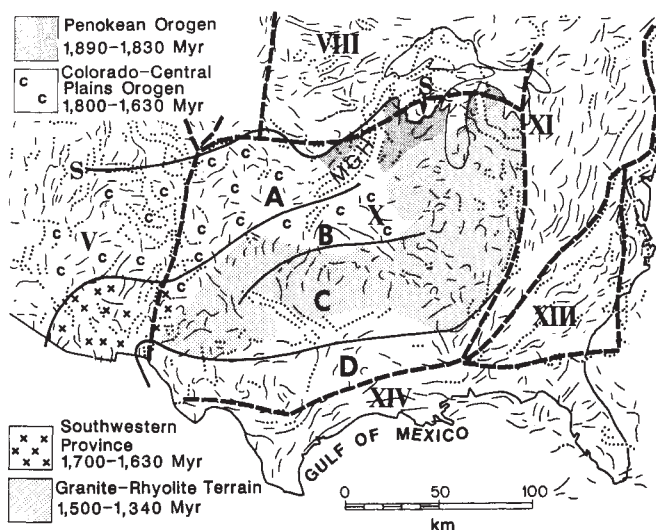


Fig. 3 Buried Proterozoic orogenic belts in the south-central United States and their relationship to gravity trends in Domains V and X. Line labelled S marks southern limit (buried west of MGH = Midcontinent Gravity High) of Archean and Trans-Hudson orogen. ENE-trending belts A (1,780-1,690 Myr), B (1,680-1,610 Myr), C ($\leq 1,470$ Myr) and D ($\leq 1,170$ Myr) between solid lines are after Anderson¹⁵. Geology illustrated by patterns is from Bickford *et al.*¹⁶.

between structural provinces interpreted as collisional sutures, such as Superior-Churchill¹⁰. Some gravity domain boundaries, however, do not correspond to well-known structural boundaries. For example, large gradient magnitudes occurring along the boundary between gravity domains II and VI suggest that it ranks in tectonic importance with known structural province boundaries, and provide further evidence that it too is a possible suture^{9,11}, marking a fundamental subdivision within the Churchill Province.

The most common pattern of both intra-domain trends and domain boundaries is subparallel NNW to NNE or ENE to NE (Fig. 2). If the corresponding Precambrian structural province boundaries are viewed as the product of Proterozoic Wilson cycles¹⁰, then the gravity domain boundaries reflect an ancient pattern of rifting, reassembly and growth imposed on an Archean supercontinent. This pattern contradicts an earlier interpretation of a proposed concentrically-zoned pattern of Bouguer gravity anomalies centred within the western Superior Province as a signature of a pre-2,000 Myr, large-scale meteorite impact that controlled the siting of Proterozoic fold belts¹². The concentric pattern is not evident in the gradient trends, supporting the argument that whatever concentric pattern may be apparent in Bouguer gravity reflects essentially an isostatic response to long wavelength topographic variations¹³.

In the past, continental assembly and growth history has been determined by estimating the time of crustal generation or stabilization by isotopic dating. In Australia, relative ages of gravity domains based on a Bouguer anomaly contour map have been inferred where gravity trends on one side of a domain boundary are parallel to it and on the other side are oblique¹⁴, the underlying assumption being that oblique and parallel trends probably predate and postdate, respectively, formation of the boundary. Relative ages determined in this fashion were substantiated by absolute ages of the first deformations in the corresponding orogenic domains. In general, we find the same relationship for gravity trends and ages can be understood within the context of collisional plate tectonics. A passive margin involved in collision will retain its inherent pre-collisional pattern of structural and, thus, gravity trends but the active margin will be characterized by structures, with resultant gravity expression, parallel or subparallel to the collision boundary and by younger ages. A pattern of crustal accretion, as defined by gravity trends, indicates that the post-Archean assembly of the North American continent has progressed essentially outward from the Archean Superior (VIII) and Wyoming (VI) provinces (Fig. 2). The Slave Province, located within II, formed another

centre of growth but this is not strongly expressed in the gravity trend map.

The buried Precambrian basement in south-central North America, investigated by drill-holes and extrapolating data from limited outcrops, is viewed as comprising three or four roughly E-W orogenic belts lying to the south of the Wyoming and Superior provinces^{7,15-17} (Fig. 3). These belts transect gravity domains V and X and are successively younger southward, ranging from about 1,800 to 1,100 Myr. Thus, whereas isotopic dating indicates that the outward pattern of growth of the North American continent is maintained in these belts, the association of the boundaries of these orogenic belts with strong gravity trend signatures is not. Indeed, domain X, within which the greater parts of the belts lie, has a diversity of gravity trends (Fig. 3). A favoured geological-based model for the development of these orogens is a progressively southward-migrating continental-margin arc system associated with a northward-dipping subduction zone⁷. The lack of strong gravity signatures within and along the orogen boundaries may, therefore, be consistent with the suggested absence of collision tectonics in their evolution and, thus, the migratory model.

The wide distribution of gravity data on many continents provides a powerful means for investigating continental structure and evolution. The present study provides one more example demonstrating that the utility of gravity data may be further improved by reformatting conventional Bouguer anomaly data into horizontal gravity gradients.

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Mechanical energy budget of a fold-and-thrust belt

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Recent studies^{1,2} have shown that an active fold-and-thrust belt is mechanically analogous to the wedge of soil or snow that forms in front of a moving bulldozer; such a wedge attains a critical taper in which the regional state of stress is everywhere on the verge of brittle failure. Here this critical-taper model of the regional stress field is combined with a simple kinematic description of the steady-state deformation of an active, eroding fold-and-thrust belt, to examine the mechanical energy balance of brittle, frictional mountain building. Part of the work performed by the compressive stress at the rear of a fold-and-thrust belt is dissipated by frictional heating on the décollement fault, part is dissipated by internal frictional processes that accompany the brittle deformation within the critically tapered wedge, and part is expended against gravitational body forces. The total power expenditure in the steady-state fold-and-thrust belt of western Taiwan is about five gigawatts, of which 45% is being dissipated against basal friction, 39% is being expended against gravity and only 16% is being dissipated internally.

The magnitude of the critical taper in an active fold-and-thrust belt is governed by the relative magnitudes of the basal sliding friction and the Coulomb strength of the wedge material; increasing the basal traction increases the taper, whereas increasing the wedge strength decreases it. Erosion exerts a significant control on the mechanics of a fold-and-thrust belt because an eroding wedge must continually deform to maintain its critical taper. An active fold-and-thrust belt in unchanging tectonic and climate conditions can attain a dynamic steady state in which the influx of freshly accreted material at the toe is balanced by the erosive efflux off the top.

The idealized cross-sectional geometry of a steady-state, critically tapered fold-and-thrust belt is shown in Fig. 1. Deformation is presumed to be occurring within a trapezoidal segment of a wedge overlying a décollement fault; the left margin of this wedge is the toe or deformation front, where an undeformed sedimentary section of thickness h is being accreted, and the right margin is the rear, where deformation ceases. Let α denote the constant surface slope of the wedge topography, and let β denote the constant dip of the décollement fault. The thin-skinned nature of foreland fold-and-thrust belts justifies the approximation that the taper of the wedge is small; that is, $\alpha + \beta \ll 1$. We adopt a system of Cartesian coordinates, with x aligned along the top surface of the wedge and z pointing obliquely down; the origin is taken to be at the buried wedge vertex, so the distance to the deformation front in the small-angle approximation is $x_0 = h(\alpha + \beta)$.

Let $\mu = \tan \phi$ be the coefficient of internal friction of the wedge material, let μ_b be the coefficient of sliding friction along the base, and let λ and λ_b be the average ratios of pore-fluid pressure to lithostatic pressure³ in the wedge and on the base. Assuming these four quantities governing the strength are constant, the state of stress in a thin-skinned, critically tapered wedge is given by⁴

$$\sigma_{xx} = \sigma_1 = -\Lambda \rho g z; \quad \sigma_{zz} = \sigma_3 = -\rho g z; \quad \sigma_{xz} = \rho g \alpha z \quad (1)$$

where ρ is the constant wedge density and $\Lambda = 1 + 2(1 - \lambda) \sin \phi / (1 - \sin \phi)$. This satisfies the narrow-taper static equilibrium equations and the free-surface boundary conditions on top of the wedge; it also satisfies the Coulomb⁵ failure criterion $\bar{\sigma}_1 / \bar{\sigma}_3 = (1 + \sin \phi) / (1 - \sin \phi)$, where $\bar{\sigma}_1 = \sigma_1 + p_f$ and $\bar{\sigma}_3 = \sigma_3 + p_f$ are the effective principal stresses, and $p_f = \rho g z$ is the pore-fluid

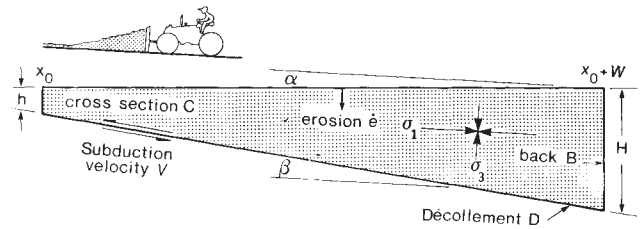


Fig. 1 Schematic cross-section of a critically tapered steady-state fold-and-thrust belt, indicating some of the terminology used in the derivation.

pressure. The basal boundary condition that the shear traction $\tau_b = \sigma_{xz} - (\alpha + \beta)(\sigma_1 - \sigma_3)$ must be equal to the frictional resistance $\mu_b(1 - \lambda_b)\rho g z$ is satisfied provided that⁴

$$\alpha + \beta = \frac{\beta + (1 - \lambda_b)\mu_b}{\Lambda} \quad (2)$$

This equation gives the critical taper $\alpha + \beta$ of a thin-skinned Coulomb wedge, in terms of its décollement dip β and its mechanical properties μ , μ_b , λ and λ_b .

Let u and v denote the x and z components of the steady eulerian velocity within the wedge, and let V denote the down-dip subduction velocity of the rigid lower plate. Ignoring terms of order $\alpha + \beta$, the cross-sectional accretionary influx rate at the deformation front is then hV , and the steady-state width W is given by

$$hV = \epsilon W \quad (3)$$

where ϵ is the constant erosion rate off the top. We ignore any compaction of the wedge material after accretion and regard the flow u , v as incompressible; this assumption is consistent with the assumed constancy of the density ρ in the critical taper model, and it should be a good approximation whenever the entering sediments are reasonably well lithified. In the small-angle approximation, the predominantly horizontal velocity u is independent of depth, and thus completely determined by the thin-skinned mass balance condition $hV = \epsilon(x - x_0) + ux(\alpha + \beta)$. The remaining component v can then be obtained from the continuity equation $\partial u / \partial x + \partial v / \partial z = 0$ and the basal boundary condition $v = u(\alpha + \beta)$ on $z = x(\alpha + \beta)$, with the result

$$u = \frac{-\epsilon + hVx^{-1}(1 + x_0/W)}{\alpha + \beta} \quad (4)$$

$$v = -\epsilon + \frac{hVzx^{-2}(1 + x_0/W)}{\alpha + \beta}$$

Mass balance is seen to constrain the large-scale deformation completely; an entering rock that is accreted with velocity V at the toe decelerates and is shortened horizontally, in addition to being uplifted in response to the erosion. The rear of the fold-and-thrust belt is fully affixed to the overriding plate because $u = 0$ there, and the normal velocity v on the top is everywhere equal and opposite to the erosion rate ϵ , as expected. The variable slip rate on the décollement fault, in the small-angle approximation, is simply $V - u$.

The equation describing the mechanical energy balance is obtained by taking the dot product of the static equilibrium equations and the velocity relative to the subducting plate, and integrating over the wedge cross-section, which we denote by C . This leads, after an integration by parts and neglect of terms of order $\alpha + \beta$, to

$$\dot{W}_B = \dot{W}_D + \dot{W}_S + \dot{W}_G \quad (5)$$

where

$$\dot{W}_B = -V \int_B \sigma_1 dz \quad (6)$$

$$\dot{W}_D = \int_D \tau_b (V - u) dx \quad (7)$$

$$\dot{W}_S = \int_C (\sigma_1 \dot{\epsilon}_1 + \sigma_3 \dot{\epsilon}_3) dx dz \quad (8)$$

$$\dot{W}_G = \rho g \int_C (u\alpha - v + V\beta) dx dz \quad (9)$$

The quantities $\dot{\epsilon}_1 = \partial u / \partial x$ and $\dot{\epsilon}_3 = \partial v / \partial z$ are the (equal and opposite) principal strain rates, and B and D denote the back and décollement surfaces of the fold-and-thrust belt. The integral over the front surface vanishes because $u = V$ there, and that over the top vanishes because it is a free surface.

The quantity $\dot{W}_B$ on the left of equation (5) is the rate at which the compressive stress does work on the back of the fold-and-thrust belt—in the bulldozer analogy this is the rate of work performed by the bulldozer—and the three terms on the right represent the three ways in which this work is either dissipated or expended. The quantity $\dot{W}_D$ is the rate of work dissipated against sliding friction on the décollement fault, and $\dot{W}_S$ is the rate of work dissipated against internal friction within the wedge. In both cases the dissipated energy manifests itself primarily as heat, although $\dot{W}_S$ also incorporates the energy required to create freshly fractured surfaces within the wedge^{6,7}. The final quantity $\dot{W}_G$ is the rate of work done in uplifting rocks against gravitational body forces; the term $u\alpha - v + V\beta$ in equation (9) is the vertically upward component of velocity relative to the lower plate. All the rates of work are measured per unit length along the strike of the fold-and-thrust belt.

Inserting the stresses (1) and velocities (4) into equation (6), we find that the total rate of work is

$$\dot{W}_B = \frac{1}{2} \rho g \Lambda V H^2 \quad (10)$$

where $H = (x_0 + W)(\alpha + \beta)$ is the thickness at the back of the fold-and-thrust belt—or in the bulldozer analogy the height of the bulldozer blade. The fractional extent to which this total work is either dissipated against décollement or internal friction or expended against gravity is found from equations (7)–(9) to be

$$\frac{\dot{W}_D}{\dot{W}_B} = \frac{(1 - \lambda_b)\mu_b}{\beta + (1 - \lambda_b)\mu_b + \Lambda(h/W)} \quad (11)$$

$$\frac{\dot{W}_S}{\dot{W}_B} = \frac{(\Lambda - 1)(h/W)}{\beta + (1 - \lambda_b)\mu_b + \Lambda(h/W)} \quad (12)$$

$$\frac{\dot{W}_G}{\dot{W}_B} = \frac{\beta + h/W}{\beta + (1 - \lambda_b)\mu_b + \Lambda(h/W)} \quad (13)$$

Taiwan is the site of an oblique collision between the continental margin of China, situated on the subducting Eurasian plate, and the Luzon arc, on the Philippine Sea plate⁸. The Central Mountains, Hsuehshan Range and western foothills in the middle of the island are an actively deforming steady-state fold-and-thrust belt characterized by² a regional décollement dip $\beta = 6^\circ$, a remarkably constant surface slope $\alpha = 3^\circ$, a pore-fluid/lithostatic pressure ratio $\lambda = \lambda_b = 0.7$ and a density $\rho = 2,500 \text{ kg m}^{-3}$. These observations are consistent with the critical taper equation (2) if we adopt common rock mechanics laboratory values^{9–11} of the basal and internal coefficients of friction, $\mu_b = 0.7$ and $\mu = 0.8$. The rate of convergence between the Eurasian and Philippine Sea plates^{12,13} is $V = 70 \text{ km Myr}^{-1}$ and the thickness of the incoming sediments at the deformation front in the western foothills⁵ is $h = 7 \text{ km}$. The mean erosion rate estimated by various methods^{14–16} is $\dot{\epsilon} = 5.5 \text{ km Myr}^{-1}$ and the width of the region of steady-state topography⁸ is $W = 90 \text{ km}$; the good agreement with equation (3) and the uniformity of the width attest to the steady-state nature of the deformation.

Evaluating equation (10) using the above physical properties and geometric and kinematic parameters, we find that the rate

of work of the regional compressive stress acting on the plate boundary in Taiwan is $\dot{W}_B = 25 \text{ kW m}^{-1}$. The length along strike of the steady-state region⁸ is $\sim 200 \text{ km}$, so the total power expenditure is about five gigawatts. This is the amount of power being supplied by the plate-tectonic heat engine to build the rugged mountains of Taiwan and maintain them against the rapid tropical erosion—it is approximately that supplied by five nuclear power plants and is in fact roughly equal to the total electrical power generation of the island¹⁷. Evaluating equations (11)–(13) we find the partition of this total rate of work to be $\dot{W}_D/\dot{W}_B = 45\%$, $\dot{W}_S/\dot{W}_B = 16\%$ and $\dot{W}_G/\dot{W}_B = 39\%$; the dominant mechanism of dissipation in Taiwan is thus frictional heating on the décollement, followed closely by work done against gravitational body forces.

Three parameters that can vary widely from one fold-and-thrust belt to another are the décollement dip β , the pore-fluid/lithostatic pressure ratio λ and the effective coefficient of basal friction $\mu_b(1 - \lambda_b)$. The influence of these three variables on the energy budget can be readily ascertained by inspection of equations (11)–(13). Increasing the décollement dip β increases the amount of work expended against gravity, because of the greater thickness of uplifted material as well as the greater upward component of velocity relative to the subducting plate. Furthermore, $\dot{W}_G/\dot{W}_B$ is always positive for any critically tapered fold-and-thrust belt whose décollement dips toward its rear; this disproves the 'gravity spreading' hypothesis¹⁸, which postulates that gravity and not a regional compressive stress is the main force acting to emplace and deform a fold-and-thrust belt. Increasing λ decreases $\dot{W}_S/\dot{W}_B$, owing to the decreased traction on the conjugate thrust faults along which failure occurs within the wedge, whereas increasing $\mu_b(1 - \lambda_b)$ increases $\dot{W}_D/\dot{W}_B$ owing to the increased basal traction.

In the limit $\dot{\epsilon}/V = h/W \ll \alpha + \beta$, equations (11)–(13) reduce to $\dot{W}_S/\dot{W}_B = 0$, $\dot{W}_D/\dot{W}_B = [(1 - \lambda_b)\mu_b]/[\beta + (1 - \lambda_b)\mu_b]$ and $\dot{W}_G/\dot{W}_B = \beta/[\beta + (1 - \lambda_b)\mu_b]$. Work done against internal friction is thus negligible in such a wide, slowly eroding fold-and-thrust belt, and frictional heating on the décollement fault is the dominant dissipation mechanism ($\dot{W}_D/\dot{W}_B > 50\%$) if $\beta < (1 - \lambda_b)\mu_b$, whereas work done against gravity is the dominant mechanism ($\dot{W}_G/\dot{W}_B > 50\%$) if $\beta > (1 - \lambda_b)\mu_b$. This illustrates a general rule that fold-and-thrust belts with moderate basal dips and moderate basal fluid pressures are dominated by work done against friction on the décollement, whereas those with steeper basal dips or nearly lithostatic basal fluid pressures are dominated by work done against gravity; internal dissipation is generally the least important mechanism even in narrow, rapidly eroding fold-and-thrust belts, as in Taiwan.

Note finally that a twofold decrease in the erosion rate doubles the steady-state width W and nearly quadruples the total rate of work $\dot{W}_B$, because of the increased cross-sectional area of the zone of deformation; a twofold increase in the subduction rate has the same effect on the steady-state width W , but it causes nearly an eightfold increase in $\dot{W}_B$ owing to the explicit factor of V —the speed of the bulldozer—in equation (10). Even though the subduction rate V plays a more important role than the erosion rate $\dot{\epsilon}$ in determining the overall amount of work required to build and maintain a steady-state fold-and-thrust belt, the partition into the percentage work dissipated against basal or internal friction or expended against gravity depends only on the ratio $\dot{\epsilon}/V = h/W$.

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The carbon-isotope shift at the Permian/Triassic boundary in the southern Alps is gradual

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Carbon isotope ratios in marine carbonate rocks have been shown to shift at some of the time boundaries associated with extinction events; for example, Cretaceous/Tertiary¹ and Ordovician/Silurian². The Permian/Triassic boundary, the greatest extinction event of the Phanerozoic³, is also marked by a large $\delta^{13}\text{C}$ depletion^{4,5}. New carbon isotope results from sections in the southern Alps show that this depletion did not actually represent a single event, but was a complex change that spanned perhaps a million years during the late Permian and early Triassic. These results suggest that the Permian/Triassic (P/Tr) extinction may have been in part gradual and in part 'stepwise'^{6,7}, but was not in any case a single catastrophic event.

Carbon isotope ratios in sea-water bicarbonate depend on the relative net fluxes of carbon into buried organic carbon of low $\delta^{13}\text{C}$ and carbonate rock of $\delta^{13}\text{C}$ equalling that of the bicarbonate^{8,9}. Micritic marine limestones generally (but not invariably) preserve their primary carbon isotope ratios because carbonate in any altering solution is dominated by the original rock, as demonstrated in a calculated model¹⁰ and verified by comparisons with organic carbon isotope ratios¹¹⁻¹³, coeval shell carbonate¹⁴, marine strontium isotope ratios^{13,15} and stratigraphically correlative carbonates¹¹⁻¹⁵. Carbon isotope drops associated with mass extinctions have been ascribed to a cessation of biological production in the surface of the 'Strangelove ocean' following a meteorite impact, allowing the surface ocean to assume the mean oceanic value of $\delta^{13}\text{C}$ (ref. 16), or alternatively to a large drop in sea level, that both reduces the shelf area in which diversity can develop and exposes the shelf (as well as the paralic zone) to erosion of its previously accumulated organic carbon¹⁷. However, the actual connection between mass extinction and carbon isotope shifts is still somewhat ambiguous.

It is therefore of considerable interest to define the exact course of the carbon isotope shift associated with a major mass extinction. Is the overall shift a single or multiple episode, and what is the rate of approach to equilibrium? In the Permian/Triassic of the southern Alps some well-preserved and rather complete sections (Figs 1, 2) across the boundary present an opportunity to investigate these questions.

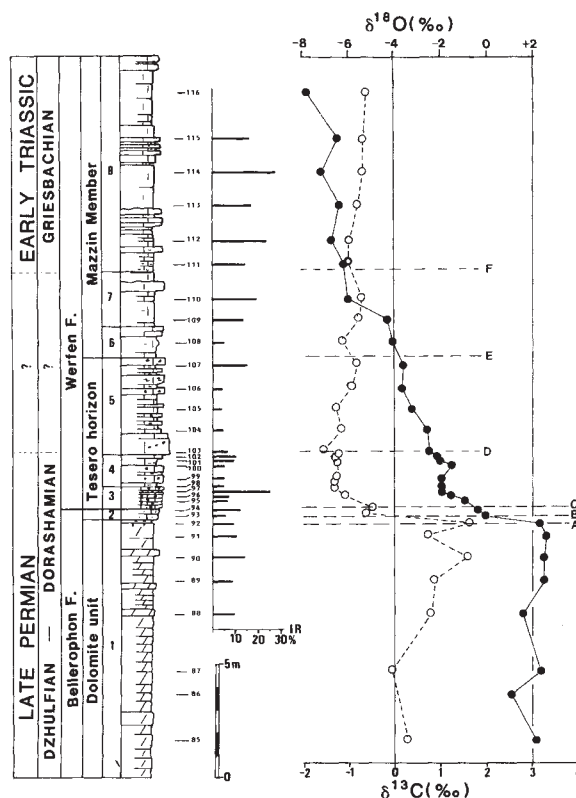


Fig. 1 Profiles of $\delta^{13}\text{C}$ (—●—), $\delta^{18}\text{O}$ (—○—), and insoluble residue (IR) across the P/Tr boundary at Tesero in the eastern Dolomite Alps of Italy. Stratigraphy based on Neri *et al.*²⁰. Important horizons are designated: A, beginning and B, end of sharp drop in $\delta^{13}\text{C}$ within the Bellerophon Formation; C, contact Bellerophon Formation with the Tesero Horizon, with a change in sedimentation; D, last Permian fossils; E, contact Tesero Horizon with the Mazzin Member; and F, lowest beds of definite Triassic aspect.

The Bellerophon Formation represents the uppermost Permian in the southern Alps (Italy, Austria), at the western end of the Tethys Sea. The section at Tesero, Italy (Fig. 1) exposes the upper part of the Bellerophon Formation as subtidal black limestone in the near-shore facies¹⁸⁻²⁰. The section at Auronzo, Italy, 80 km east of Tesero, comprises limestones of a more open marine inner shelf facies (Fig. 2). The Bellerophon is of late Permian age. Based on its foraminiferal assemblage it is assigned to the Dzhulfian-Dorashamian Stages¹⁸⁻²¹.

The Bellerophon Formation is concordantly overlain, at level C in Figs 1 and 2, by grey carbonates of the Tesero Horizon of the Werfen Formation. In the west (Fig. 1) the 6-m thickness is built up of layers of oolites 5-10 cm thick, alternating with micrite-microsparite beds of similar thickness. In the east (Fig. 2) the oolite layers are missing, leaving 1-3 m of micrite-microsparite beds. The Tesero Horizon is overlain by grey, sometimes shaly limestones of the Mazzin Member.

New palaeontology and stratigraphy¹⁸⁻²¹ suggest that the P/Tr boundary lies within the lower part of the Werfen, at Tesero somewhere above the last occurrence of Permian microfossils in the middle of the Tesero Horizon (Fig. 1D) and the first beds of definite Triassic aspect within the lower Mazzin (Fig. 1F)—probably closer to D than to F²¹. The Mazzin (as well as the upper Tesero Horizon) belongs to the Griesbachian Stage of the Lower Triassic²², the lower part of the Tesero Horizon is evidently Dorashamian. Although the P/Tr boundary in even the best of sections is generally regarded as involving a major paraconformity²³, and has been so described in the Alps²⁴, the new work¹⁸⁻²² has emphasized the continuity as well as the

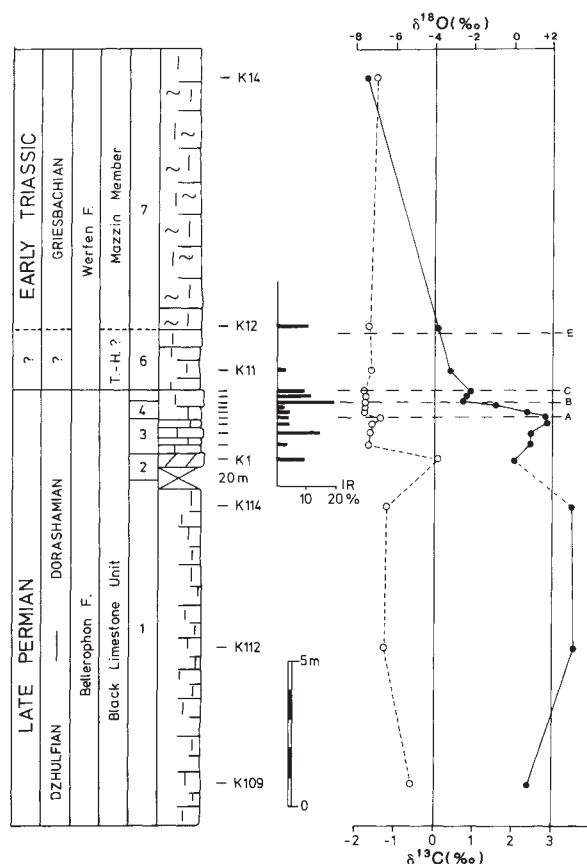


Fig. 2 Profiles across the P/Tr boundary near Auronzo in the western Carnic Alps of Italy—80 km east of Fig. 1. The upper part of the section is taken along Highway 532 2 km east of Auronzo (stratigraphy by Noé²¹); the lower part from the valley below (by S. Krumser). Horizons as in Fig. 1.

conformity of the sedimentological and palaeontological record in that region. The palaeontology is not definitive as to which biozones²⁵ are represented in the Dorashamian of the Bellerophon/lower Tesero (Noé²¹ says "... At least Lower Dorashamian age ...") and in the Griesbachian of the upper Tesero/Mazzin. But the smoothness of the carbon isotope profiles that we present below suggests a nearly continuous section.

These two sections were sampled in detail across this critical zone. Carbon and oxygen isotope ratios were determined²⁶ using a Varian 250 mass spectrometer, and reported using the conventional 'δ' notation relative to the PDB standard. Reproducibility of duplicate samples is better than 0.15%. Carbon and oxygen isotope results are displayed in Figs 1 and 2.

We direct attention first to the gradual decrease in $\delta^{13}\text{C}$ from the level B within the Permian Bellerophon, to the tops of measured sections within the Triassic Mazzin. This decrease includes the transitional beds D-F of questionable age assignment in the upper part of the Tesero Horizon and the lower part of the Mazzin. By correlation it therefore also extends across the time of final mass extinction at the P/Tr boundary²⁷. The drop of $\delta^{13}\text{C}$ is at a rate of $\sim 0.2\% \text{ m}^{-1}$ in both sections. This gradual change also seems to be unrelated to the fraction of insoluble residue (IR in Figs 1, 2) in the limestones, or to differing sedimentary facies (oolites, micritic limestone, marly dolomite). Within the Mazzin the downward slope of $\delta^{13}\text{C}$ shows a possible discontinuity just below level F (samples 109–110), but the data are not sufficient to decide whether this represents a paraconformity. Both profiles are still decreasing in $\delta^{13}\text{C}$ at the tops of the sampled sections, in the lower part of the Mazzin.

Other data on $\delta^{13}\text{C}$ in the Werfen (including the Mazzin Member) from elsewhere in the Dolomite and Carnic Alps generally lie near 0‰ (refs 28, 29), so a minimum in the profile of $\delta^{13}\text{C}$ probably lies not far above the sections shown in Figs 1 and 2.

The gradual drop is immediately preceded by a sharper drop in interval A-B at Auronzo (Fig. 2) from $\sim +3\%$ to $<1\%$. At Tesero (Fig. 1) the sharpest drop (samples 92 to 93) is from 3‰ to $<2\%$; it is not clear from this profile whether the further drop of intermediate slope, to $<1\%$ (samples 94 to 97), is a part of this event or not. The step is at the rate of more than $3\% \text{ m}^{-1}$ at both sections. $\delta^{13}\text{C}$ at earlier levels in the Dzhulfian/Dorashamian lie near 5‰ elsewhere (refs 5, 11, 29 and A.B., M.M. and W.T.H., manuscript in preparation), so the drops in $\delta^{13}\text{C}$ shown near the P/Tr boundary in these two Alpine sections are preceded by at least one other decrease in $\delta^{13}\text{C}$.

A preliminary carbon isotope profile presented by Brandner *et al.*³⁰ from a section at San Antonio, 3 km north of Auronzo, is similar to our profile at Auronzo in the overall drop displayed from the late Permian into the early Triassic. Brandner *et al.* show values of $\delta^{13}\text{C}$ near +0.5‰ for Tesero-equivalent beds at the base of the Mazzin, similar to our section, but their sampling interval was too coarse to show that the sharp drop in $\delta^{13}\text{C}$ (equivalent to our event A-B) occurred within the Bellerophon Formation, below the P/Tr boundary.

Values of $\delta^{18}\text{O}$ in the limestones (Figs 1, 2) are all negative and rather constant across lithologic, formation and age boundaries. They show no anomalies related to the shifts of $\delta^{13}\text{C}$, and the two isotope ratios are not correlated. As usual in our experience, the processes governing primary changes and secondary alteration in $\delta^{18}\text{O}$ are different from those for $\delta^{13}\text{C}$; oxygen will not be discussed further here.

These two sections in the southern Alps exhibit details of carbon isotope variations in P/Tr not previously evident in profiles from other areas^{5,11,29}. A comparable overall depletion of $\delta^{13}\text{C}$ from +3 to -2% was reported from the Shangsi and Changxing sections in China³¹. In both sections the drop began $<2 \text{ m}$ below the P/Tr boundary, and continued irregularly to at least 4 m above the boundary. The initial drop in the Chinese sections lies near the P/Tr boundary but within the Permian, analogous to our event A-B, and depletion continues through the P/Tr boundary into the Triassic, as in the upper parts of our sections. But the isotope profiles in the Alps are much more regular than those in China; this suggests that the record of sedimentation is more continuous and complete in the Alps than in China.

One expects a change in $\delta^{13}\text{C}$ in marine carbonate to respond in a very short time (10^3 – 10^4 yr) to a single step change in the ratio of organic to total flux; if the change is slow and continuous, $\delta^{13}\text{C}$ may follow it closely. Such a gradual change is recorded in the Alpine sections (Figs 1, 2), continuous through the P/Tr boundary and its final mass extinction²⁷; the carbon isotopes do not show any dramatic shift that can be associated with the final extinction. The slow slide of $\delta^{13}\text{C}$ across the P/Tr boundary corresponds to 10–20 m of carbonate sedimentation. At expected rates of sedimentation^{32,33}, this represents 0.1–2 Myr, which is consistent with the stratigraphic interval of a fraction of a stage of the late Permian or early Triassic.

The sharper drop during interval A-B, which precedes the gradual decline, is accomplished within a few tens of centimetres, in both sections. This drop possibly marks a hiatus in the sedimentary record, but the profile at Auronzo (Fig. 2) records two intermediary values of $\delta^{13}\text{C}$ between levels A and B, suggesting a continuous record. If the record is indeed continuous, the steepened change A-B could have been caused either by a radically slowed rate of sedimentation (relative to the overlying and underlying sections), or to a very rapid drop in $\delta^{13}\text{C}$. Study of thin sections does not reveal a radical difference in sedimentary facies between the interval A-B and those above and below. Consequently we are inclined to ascribe the profile to a sharp drop in $\delta^{13}\text{C}$ of sea water rather than to a sharp drop

in rate of sedimentation.

Although we cannot yet present any general model relating diminished $\delta^{13}\text{C}$ to declining biomass and decreasing diversity, these Alpine isotope profiles may be useful in guiding further palaeontological and geochemical studies. When diversity is counted by stages it had already peaked in the Guadalupian (mid-Permian), and declined in the Dzhulfian and Dorashamian to a minimum in Scythian time³. Our carbon isotope profiles suggest that detailed palaeontological studies of mass mortality and extinction should consider the possibilities of stepwise extinction⁶ in the Upper Permian, and a final gradual decline crossing the P/Tr boundary.

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Correlation of ^{210}Pb removal with organic carbon fluxes in the Pacific Ocean

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The flux of particulate organic carbon reaching the deep sea is fundamental to our understanding of the global carbon cycle. Measurements of carbon accumulation in sediments do not quantify this flux because carbon is rapidly oxidized during settling and prior to burial. Using data from material collected by particle traps, we report here a strong correlation between the fraction of ^{210}Pb removed from the ocean by particle settling and the flux of particulate organic carbon. The fraction of ^{210}Pb removed from the water column may be determined by measurements of the inventory of excess ^{210}Pb in bottom sediments as well as by measurements in sediment traps. Thus, the excess ^{210}Pb inventory in deep-sea sediments is a proxy for the amount of particulate organic carbon delivered to the sediments over the past 100 years.

Variability of primary productivity in the euphotic zone appears to control the flux variations of many elements throughout the water column¹. Even radioisotopes generated at mid-ocean depths seem to respond to the surface-derived particle flux². High correlations ($r = 0.91$ – 0.99) between the fluxes of organic carbon and various trace metals (Al, Cu, Fe, Mn, Ni, P, Pb, V, Zn) provide evidence that the settling of particulate organic matter regulates the sedimentation of these elements³. The photic zone is also a sink of atmospheric CO_2 and hence the flux of organic carbon in this zone is an important variable in determining the global CO_2 inventory.

We have measured seasonal fluxes of ^{210}Pb and organic carbon⁴ at four Manganese Nodule Project (MANOP) sites in the Pacific Ocean⁵. ^{210}Pb ($t_{1/2} = 22.3$ years) is generated throughout the water column by ^{226}Ra decay. Small amounts of ^{210}Pb are

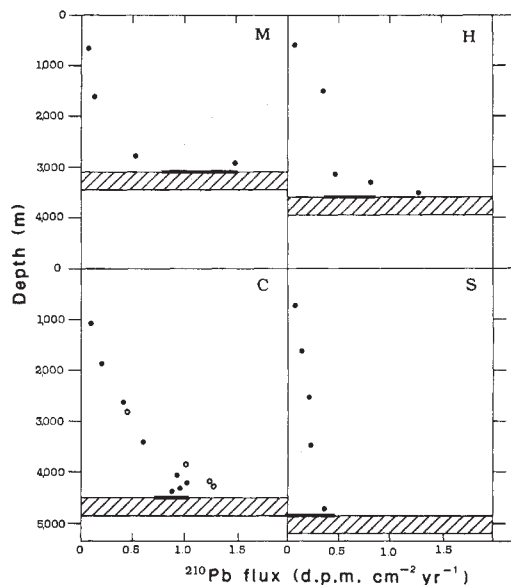


Fig. 1 ^{210}Pb flux increases with depth at each station but at quite different rates. The different symbols at C represent repeated experiments two years apart. The dark bar in the hatched area represents the flux of ^{210}Pb necessary to support the inventory of excess ^{210}Pb measured in the sediment by Cochran⁷ and W. S. Moore (unpublished data). The length of the bar illustrates variable ^{210}Pb accumulation. Sites M ($8^{\circ}49' \text{N}$, 104°W , depth = 3,100 m) and H ($6^{\circ}30' \text{N}$, 93°W , depth = 3,570 m) were sampled between September 1980 and October 1981. Site S (11°N , 190°W , depth = 4,620 m) was sampled between December 1982 and October 1983. Site C (01°N , 139°W , depth = 4,450 m) was sampled between December 1982 and October 1983 and between February 1984 and April 1985.

also derived from atmospheric deposition. Deficiencies of dissolved ^{210}Pb with respect to ^{226}Ra in the deep Pacific demonstrate that ^{210}Pb is being removed on a time scale that is comparable to its mean life⁶. Our measurements reveal increased particulate fluxes of ^{210}Pb with depth (Fig. 1) as expected from measurements in the water column. ^{210}Pb removed in the particle flux

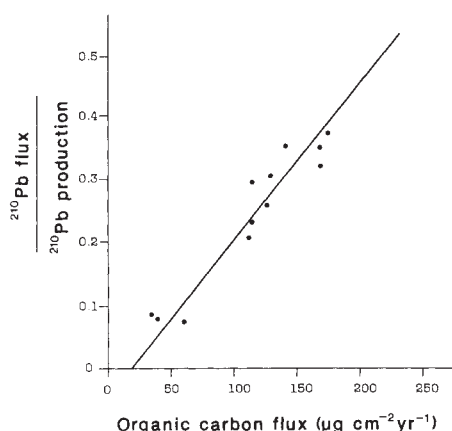


Fig. 2 Average annual organic carbon flux correlates strongly with the ratio of the ^{210}Pb flux to each trap divided by the annual production (including atmospheric deposition) of ^{210}Pb in the water column above the trap. For traps at depth greater than 3,000 m, the correlation is 0.95.

defines an inventory of excess ^{210}Pb in the underlying sediments. Such inventories may be quantified by carefully collecting deep-sea cores to preserve the sediment-water interface and measuring the difference between ^{210}Pb and ^{226}Ra through the benthic mixed layer. Possible differential short-term accumulation may require sampling of several cores at each site to establish the sediment inventory. ^{210}Pb fluxes measured near the sea floor using sediment traps agree well with inventories of excess ^{210}Pb measured in box cores at each site⁷, except at sites H and M where a large resuspension flux has been demonstrated to affect the flux to near-bottom traps⁸. At these sites comparisons to fluxes above the resuspension zone are in good agreement with sediment inventories. An important implication of this observation is that ^{210}Pb is predominantly introduced to the bottom by large particle settling, and it is conservatively buried without significant recycling at the sediment-water interface. Thus ^{210}Pb fluxes may be derived from measurements of excess ^{210}Pb inventories in sediment cores if enough high quality data are available to establish such inventories.

Only at site S do organic carbon fluxes exhibit a monotonic decrease with depth that is expected by recycling of carbon during the settling of biogenic particles through the water column. Mid-depth (~1,500 m) peaks in flux are found at sites C and H, while at site M organic carbon flux increases with depth. These features are common in other data sets but are poorly understood. Particle aggregation/disaggregation⁹, zooplankton feeding dynamics¹⁰ and chemosynthetic carbon production¹¹ have been suggested to explain these patterns.

Because ^{210}Pb fluxes exhibit relatively smooth increases with depth, there is poor correlation between organic carbon and ^{210}Pb fluxes. Consequently, the two do not appear to be directly coupled. However, ^{210}Pb is generated throughout the water column by ^{226}Ra decay, and in the open ocean only a fraction of the production is removed locally⁶. If that removal is linked to organic carbon flux, there should be a correlation between the fraction of ^{210}Pb production which is removed by particle settling and the organic carbon flux. The fraction of ^{210}Pb removed in the particle flux is the flux of ^{210}Pb as recorded in

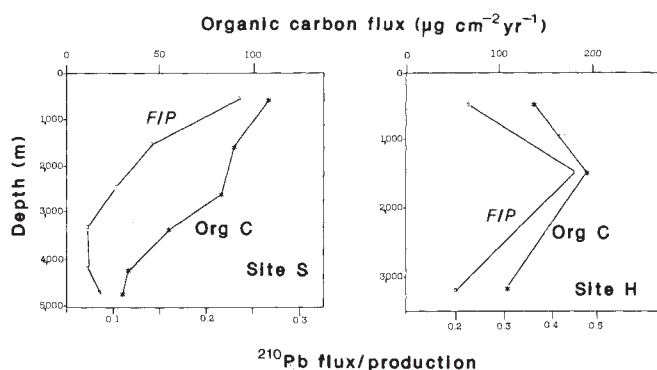


Fig. 3 The pattern of the F/P ratio tracks that of organic C flux quite closely at sites H and S.

the sediment trap (F) divided by the production of ^{210}Pb in the water column above the trap (P). The production term is derived from the ^{226}Ra content of the water above the trap^{12,13} and the expected atmospheric deposition of ^{210}Pb , 0.06 d.p.m. $\text{cm}^{-2} \text{yr}^{-1}$ at sites S and C and 0.03 d.p.m. $\text{cm}^{-2} \text{yr}^{-1}$ at sites M and H (ref. 14). The F/P ratio is the fraction of ^{210}Pb scavenged in the particulate flux.

In Fig. 2 we compare the average annual F/P ratio with the average annual organic carbon flux measured in each trap. To eliminate the poorly understood upper level effects and the near bottom resuspension effects, we restrict this comparison to traps >3,000 m deep, but above the resuspension zone.

This comparison of the F/P ratio with organic carbon flux has a correlation coefficient (r) of 0.95. If we include all of the traps above the resuspension zone, r decreases to 0.79. We believe this correlation has important implications. It does not appear to be equivalent to the strong correlations between organic carbon and lithogenic elements such as Al, Ti and Th which probably represent nondiscriminant filtering of clays and other aluminosilicates by zooplankton. This process transforms fine particles into rapidly settling fecal pellets and results in excellent correlations with carbon at a single site and single depth but poor correlations for multi-site or multi-depth data (J.D., unpublished data). For example, r between Al and organic carbon for the samples below 3,000 m reported here is 0.44. Consequently, we suggest the good correlation between the fraction of ^{210}Pb removed and organic carbon flux is a result of active Pb scavenging by organic matter. Thus, any process which enhances or diminishes the flux of carbon will enhance or diminish the ^{210}Pb flux. The strong link between the two species is demonstrated in Fig. 3, which shows two very distinct patterns of carbon flux against depth for sites H and S, and yet, the pattern of ^{210}Pb F/P with depth tracks that of carbon in each case.

The relationship between the particulate organic carbon flux (in $\mu\text{g cm}^{-2} \text{yr}^{-1}$) and F/P based on the >3,000 m traps is

$$\text{Flux } C_{\text{org}} = 8.5 + 430(F/P)$$

The only other area where we may test this model using published sediment trap data is the Sargasso Sea where ^{210}Pb and organic carbon fluxes are fairly well known through the work of Deuser, Bacon, Brewer and colleagues^{2,15}. An additional site where ^{210}Pb and organic carbon fluxes have been published is

Table 1 Comparison of predicted and measured carbon fluxes in sediment traps

Location	F/P	Organic C flux ($\mu\text{g cm}^{-2} \text{yr}^{-1}$)			Ref.
		predicted	measured	predicted/measured	
Sargasso Sea, average of 7 measurements at 3,200 m	0.15	73	83	0.88	2
Sargasso Sea, 1 trap measurement at 4,000 m	0.15	73	74	0.99	15

the Parflux E site east of Barbados¹⁵; however, the short duration of this trapping experiment limits its usefulness in establishing mean fluxes.

The predicted carbon fluxes (Table 1) are within 12% of the measured values. Thus, the correlation seems to apply to the Atlantic as well as the Pacific Ocean.

The one-dimensional model discussed thus far considers only ²¹⁰Pb derived from atmospheric deposition or ²²⁶Ra decay in the water directly above the trap (our term *P*). Another source of ²¹⁰Pb is water moving through the area that carries ²¹⁰Pb produced elsewhere. A complete ²¹⁰Pb balance in a defined volume above each trap is described as follows:

$$\text{input of } ^{210}\text{Pb} = P + V_{\text{in}}C_{\text{in}}$$

$$\text{loss of } ^{210}\text{Pb} = F + \lambda C_{210}Ah + V_{\text{out}}C_{210}$$

where V_{in} = the volume of water moving into a defined area above the trap and equals V_{out} , the volume moving out; C_{in} = the ²¹⁰Pb activity in V_{in} ; and C_{210} = the ²¹⁰Pb activity in the defined volume above the trap, λ is the decay constant of ²¹⁰Pb, A is the defined area and h is the depth of the trap. F and P (including atmospheric input) have been defined earlier.

At steady state the input and loss terms are equal, so we may simplify the ²¹⁰Pb balance to:

$$P - F = V_{\text{in}}(C_{210} - C_{\text{in}}) + \lambda C_{210}Ah$$

If the activities of ²¹⁰Pb are constant in the water passing into and out of the area over the trap, this reduces to the one-dimensional case:

$$P - F = \lambda C_{210}Ah$$

If we neglect atmospheric input, P is a function of ²²⁶Ra

$$P = \lambda C_{226}Ah$$

where C_{226} is the activity of ²²⁶Ra. Thus:

$$F/P = 1 - \frac{C_{210}}{C_{226}}$$

and we should be able to derive F/P from the activity ratio of ²¹⁰Pb/²²⁶Ra in the water above the trap. This offers another way to deduce organic C fluxes.

These simplified relationships will not apply when waters flow across gradients of production that cause changes in the flux of organic C. In this case the water entering and leaving an area would be expected to have different activities of dissolved ²¹⁰Pb.

One area where this has been well documented is the Santa Barbara Basin off southern California where ²¹⁰Pb sediment inventories¹⁶ and the fluxes of ²¹⁰Pb recorded in sediment traps¹⁷ are many times greater than the production rate (including atmospheric deposition) in the basin waters. Clearly the advection of water through the basin carries dissolved ²¹⁰Pb into the basin where ²¹⁰Pb is stripped, probably in response to high productivity in the coastal waters. It is interesting to note that about seven times more ²¹⁰Pb is deposited in the basin than is produced there and that the organic carbon flux is about 10 times greater than in the open ocean¹⁷. Another region where ²¹⁰Pb fluxes are much higher than *in situ* production plus fallout is the continental shelf of Washington state¹⁸. Similar transport must occur there.

The model outlined above assumes steady state, an assumption that may not be valid. There is abundant evidence for significant changes in productivity over a range of time scales. The residence time of ²¹⁰Pb varies from a year or two in near surface waters to tens or hundreds of years in the deep sea⁶. Achieving a steady state in such a system is unlikely. Our data show that comparisons of annual mean fluxes reduce the scatter significantly from the seasonal samples. However, we have not considered longer time-scale variability. Because the sediment inventory of ²¹⁰Pb integrates the flux over a 100-year period, this approach to defining the organic C flux to the bottom should

be less subject to breakdown of the steady-state assumption.

If future studies confirm the interpretations we have made, excess ²¹⁰Pb inventories in surficial deep-sea sediments can be used to calculate F/P and to estimate the rain rate of organic carbon to the sediment. The rain rate coupled with measurements of organic carbon burial rates defines the rate of recycling of organic carbon at the sediment-water interface. Both rates could be inferred by measurements in surficial sediments without the complexities of deploying sediment traps in the deep sea. But the technique must first be tested by comparing ²¹⁰Pb and organic carbon fluxes from other regions.

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Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic

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An interesting oceanographic problem concerns the excess major plant nutrients (PO_4 , NO_3 , SiO_3) occurring in offshore surface waters of the Antarctic¹⁻³ and north-east Pacific subarctic Oceans⁴. In a previous study⁵, we presented indirect evidence suggesting that inadequate Fe input was responsible for this limitation of growth; recently we had the opportunity to seek direct evidence for this hypothesis in the north-east Pacific subarctic. We report here that the addition of nmol amounts of dissolved iron resulted in the nearly complete utilization of excess NO_3 , whereas in the controls—without added Fe—only 25% of the available NO_3 was used. We also observed that the amounts of chlorophyll in the phytoplankton increased in proportion to the Fe added. We conclude that Fe deficiency is limiting phytoplankton growth in these major-nutrient-rich waters.

Water, with its resident phytoplankton, was collected from a depth of 20 m at Ocean Station 'PAPA' (50.0° N; 145.0° W), using ultra-clean trace-element sampling techniques (30-litre Go-Flo bottles on Kevlar line; samples processed inside a positive pressure portable laboratory)^{6,7}. Aliquots of the raw water were placed in 2-l acid-washed polycarbonate bottles, to which various amounts of unchelated Fe (1,000 p.p.m. Fe in HNO_3 , diluted appropriately) were added (Table 1, Fig. 1). In the oxygenated seawater (pH 8), the Fe was presumed to be in the ferric (III) state in the form of hydroxy complexes⁸. Additional methodological details will be published elsewhere.

Chlorophyll concentrations remained fairly constant for all treatments on days 1-3 (Table 1, Fig. 1). On day 4, however,

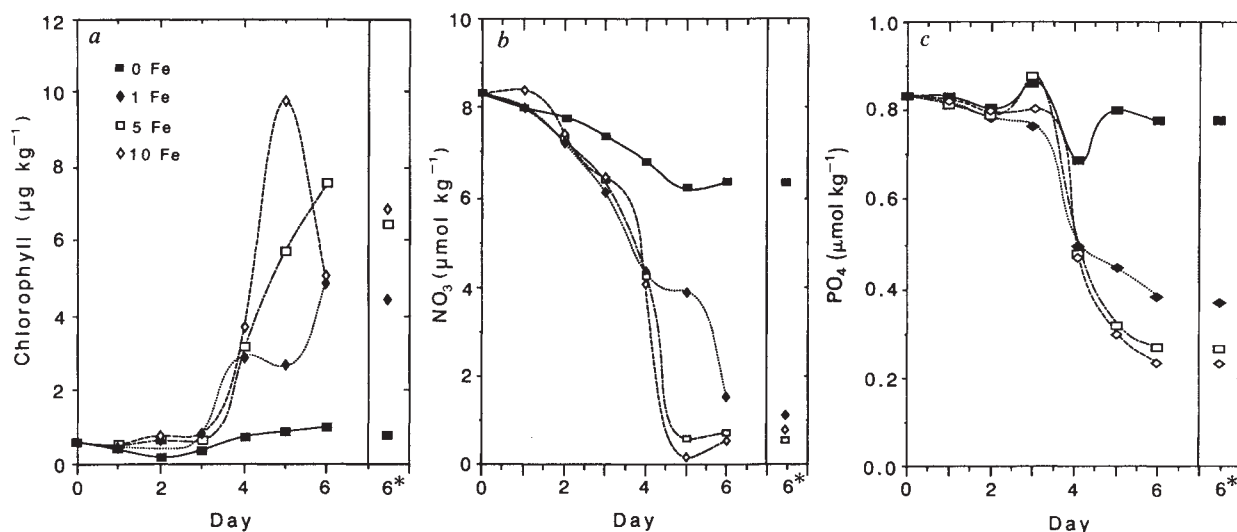


Fig. 1 Chlorophyll concentrations versus experimental day (a) in control (no added Fe) and experimental bottles with 1 nmol Fe, 5 nmol Fe and 10 nmol Fe added per kg. The data shown for day 6* represent a second set of replicates that were opened only upon the completion of the experiment. Nitrate (b) and phosphate (c) concentrations versus day are also shown. Because of the unintentional addition of $3 \mu\text{mol NO}_3 \text{ kg}^{-1}$, the data for the experimental bottles with 10 nmol Fe plus 1.0 nmol Mn and 0.1 nmol Co per kg are not included in this figure (see Table 1). Bottles were placed in three plastic bags and maintained in an all plastic deck-top incubator. Light levels were $\sim 20\%$ of those at the sea surface; temperatures were kept at $\sim 14^\circ\text{C}$ via running seawater.

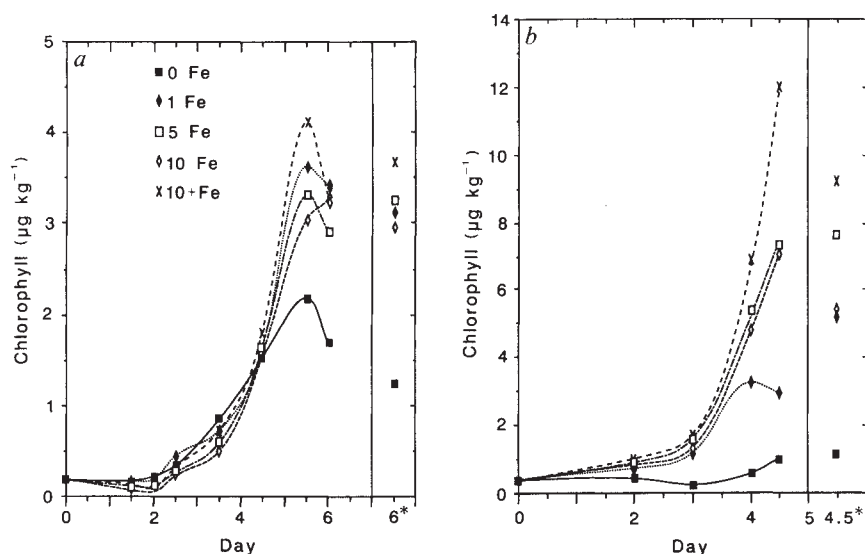


Fig. 2 Chlorophyll concentrations versus experimental day at 45.5°N ; 142.89°W (a) and at 55.5°N ; 147.5°W (b) in control (no added Fe) and experimental bottles with 1 nmol Fe, 5 nmol Fe, 10 nmol Fe, and 10 nmol Fe plus 1.0 nmol Mn and 0.1 nmol Co per kg. Day 6* and day 4.5* data represent second sets of replicates that were opened only at the end of the experiment. Initial conditions in a (15 m depth, 3 August 1987) = $0.18 \mu\text{g}$ chlorophyll kg^{-1} , 6.43, 0.70 and $10.2 \mu\text{mol kg}^{-1}$ of NO_3 , PO_4 and SiO_3 , respectively. Initial conditions in b (30 m depth, 11 August 1987) = $0.35 \mu\text{g}$ chlorophyll kg^{-1} , and 7.53, 0.88 and $23.06 \mu\text{mol kg}^{-1}$ of NO_3 , PO_4 and SiO_3 , respectively.

rapid growth began and substantial chlorophyll increases were observed in all of the bottles with added Fe. Accelerated growth continued into day 5 and appeared to peak in the 10 and 10+ (10 nmol Fe with 1.0 nmol Mn and 0.1 nmol Co kg^{-1} added) experimental bottles. Similar chlorophyll concentrations were found in replicate experimental bottles opened for the first time on day 6. In contrast, both control bottles had very low chlorophyll concentrations (1.07 and $0.85 \mu\text{g kg}^{-1}$) on day 6 that were scarcely higher than the initial day 0 values ($0.69 \mu\text{g kg}^{-1}$).

Nearly identical NO_3 uptake was observed in the 1, 5 and 10 nmol added Fe kg^{-1} bottles on days 1–4. On day 5 substantial NO_3 depletion continued in the 5 and 10 nmol Fe bottles, although it appeared to slow in the 1 nmol Fe bottle (Fig. 1, Table 1). Once again, the control bottles with no added Fe were completely different. The bottle sampled each day showed very slow NO_3 utilization and at the end of the experiment on day 6, 75% of the NO_3 originally present remained. The concentration in the other replicate initially opened on day 6 was nearly identical (6.38 versus $6.34 \mu\text{mol NO}_3 \text{ kg}^{-1}$). Much the same uptake pattern was also observed for PO_4 (Fig. 1). In the case

of SiO_3 , however, hardly any differences were observed between bottles (Table 1). This was surprising because scanning electron microscope examinations showed substantial diatom (*Nitzschia* spp.) growth in the experimental bottles, in contrast to the controls, which had abundant coccoliths but few diatoms.

Similar experiments were also performed 625 km north-west (55.5°N ; 147.5°W) and 625 km south-east (45.0°N ; 142.89°W) of Ocean Station 'PAPA' (50.0°N ; 145.0°W). Essentially the same results were found; chlorophyll data are shown in Fig. 2. These data, together with those in Fig. 1, provide consistent evidence that Fe limits phytoplankton growth in the north-east Pacific subarctic. With appropriate independent confirmation, this area could become a classic marine example of Liebig's law of the minimum⁹. This research also exemplifies the necessity of using ultraclean techniques, without which the control bottles would have had enough contaminate Fe to stimulate growth and make the control results indistinguishable from those with intentionally added Fe.

The essential roles of Fe in living systems are well known¹⁰, and it is not surprising that chlorophyll levels increase with

Table 1 Nutrient uptake and chlorophyll production by phytoplankton from 20 m depth

Day	Fe added (nmol kg ⁻¹)				
	0	1	5	10	10+*
Chlorophyll (µg kg ⁻¹)					
0	0.69 (initial value)				
1	0.49	0.46	0.52	0.46	0.49
2	0.35	0.68	0.67	0.77	0.67
3	0.41	0.84	0.67	0.84	0.92
4	0.77	2.98	3.31	3.85	3.65
5	0.92	2.77	5.97	10.16	9.09
6	1.07	5.21	7.91	5.26	6.95
6‡	0.85	4.63	6.81	7.17	14.70
NO ₃ (µmol kg ⁻¹)					
0	8.37 (initial value)				
1	8.04	7.94	8.03	8.37	11.43†
2	7.72	7.19	7.22	7.34	10.58
3	7.34	6.10	6.40	6.40	9.75
4	6.80	4.32	4.24	4.04	7.57
5	6.25	3.84	0.60	0.15	2.69
6	6.34	1.51	0.75	0.50	0.52
6‡	6.38	1.14	0.58	0.77	0.56
PO ₄ (µmol kg ⁻¹)					
0	0.83 (initial value)				
1	0.83	0.82	0.83	0.83	0.83
2	0.80	0.78	0.79	0.79	0.79
3	0.86	0.76	0.86	0.80	0.80
4	0.68	0.50	0.48	0.47	0.48
5	0.80	0.44	0.32	0.30	0.33
6	0.78	0.38	0.27	0.23	0.09
6‡	0.74	0.42	0.22	0.23	0.07
SiO ₃ (µmol kg ⁻¹)					
0	16.13 (initial value)				
1	16.94	16.96	16.88	17.01	17.04
2	17.24	17.22	17.19	16.86	17.14
3	19.49	18.51	18.53	18.08	17.96
4	18.40	17.65	17.21	17.40	16.65
5	18.80	—	16.90	15.82	15.81
6	—	—	17.31	15.82	14.43
6‡	—	18.60	—	15.34	11.69

The daily chlorophyll and nutrient data are from control and experimental bottles with 0, 1, 5 and 10 nmol added Fe kg⁻¹ at Ocean Station PAPA (50.0° N; 145.0° W): water was collected on 6 August 1987 from depth of 20 m. Data from a second set of replicates opened only at end of experiment are also shown.

* 10+ = 10 nmol Fe + 1 nmol Mn + 0.1 nmol Co kg⁻¹.

† About 3 µmol NO₃ kg⁻¹ inadvertently added with Co.

‡ The second replicate that was not opened until day 6.

increased Fe, as this element is required for the synthesis of this pigment¹¹. Iron is also required for the synthesis of several photosynthetic electron transport proteins and for the reduction of CO₂, SO₄ and NO₃ during the photosynthetic production of organic compounds¹².

In addition to the necessity for Fe at the cellular level, it is possible that oceanic Fe availability may be important in determining global atmospheric CO₂ levels and, hence, affecting global climate. The major role of the Antarctic Ocean in the removal of CO₂ from the atmosphere has been noted in several studies^{13–17}. It is argued that the lower ice age atmospheric CO₂ levels (200 p.p.m.v.) measured in ancient air^{18–21} trapped in ice resulted from the increased biological productivity and CO₂ removal at high latitudes. When high-latitude surface nutrients are completely used up by the phytoplankton, the biological removal mechanism operates at maximum efficiency and CO₂ is withdrawn from the atmosphere^{14,15}. In contrast, when surplus nutrients are left unutilized, the biological pump is inefficient and CO₂ levels increase. The range in these two extremes results in a factor of three difference in the atmospheric CO₂ content¹⁶.

We believe that a situation similar to that in the Gulf of Alaska also exists in offshore Southern Ocean waters, where surface concentrations of major nutrients such as NO₃ (25.2 µmol kg⁻¹), PO₄ (1.68 µmol kg⁻¹) and SiO₃ (54.9 µmol kg⁻¹) are 2–3 times higher (examples from GEOSECS Station 287; 2 m depth, 69°5'S; 173°30'W)²². We postulate⁵ that Fe supplies from local Antarctic continental margin sources are rapidly used up in the highly productive neritic waters of this region and little, if any, Fe mixes out into the open Southern Ocean. Also, atmospheric Fe input is limited, as Southern Ocean dust fluxes between 40° S and the Antarctic continent are among the lowest in the world because of the absence of land masses; that is, only the tip of South America and the southern island of New Zealand are in this region of strong prevailing westerly winds²³. This low dust input is exemplified by the small amounts of Al found in present-day interglacial Antarctic Vostok ice core samples. De Angelis *et al.*²⁴ report that mean Al concentrations are 2.5 ± 0.9 ng g⁻¹ during the present interglacial (Holocene) and are not significantly different from those of the previous interglacial (3.0 ± 1.5 ng Al g⁻¹, 127 kyr BP). This would be ~0.03–0.04 nmol of Fe g⁻¹ based upon Taylor's²⁵ crustal abundance estimates (8.23% Al and 5.63% Fe; Fe:Al ratio = 0.68). Levels of CO₂, in the same Vostok ice core, were highest during these interglacials, on the order of 270–280 p.p.m.v.²¹.

In contrast to interglacials, glacial periods had increased amounts of atmospheric dust Al and Fe and low CO₂ concentrations; for example, during the last glacial maximum, when Al levels were 50 times higher (~125 ng Al g⁻¹)²⁴, CO₂ concentrations were <200 p.p.m.v.²¹. The larger glacial period Al amounts are expected as tropical arid zones were five times larger, wind speeds 1.3–1.6 times higher, and atmospheric dust loads 10–20 times greater during the ice ages²⁶. We postulate that the enhanced supply of Fe from the atmosphere stimulated photosynthesis, which led to the drawdown in atmospheric CO₂ levels during glacial maxima.

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Quantitative analyses of the early angiosperm radiation

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Although the pattern of the early angiosperm radiation has been substantially clarified by palaeobotanical and stratigraphic investigations over the last 30 years¹⁻³, knowledge of associated vegetational and floristic change remains limited. Quantitative analyses of Cretaceous fossil floras indicate that by ~90 million years before present (BP) angiosperms had achieved widespread floristic dominance, at least in the Northern Hemisphere. Although some non-angiosperm plant groups show no pronounced shifts in diversity in association with the angiosperm radiation, others show marked declines that contribute to profound alterations in the composition of terrestrial plant communities. Comparative trends in systematic diversity suggest competitive displacement of cycadophytes and pteridophytes by angiosperms, but current evidence is not sufficient to exclude the probability of a more complex basis for mid-Cretaceous floristic change.

Most assessments of floristic and vegetational changes associated with the angiosperm radiation have been based either on palynological data^{4,5} or on inferences from local stratigraphic sections². Our analysis was designed as a more comprehensive attempt to quantify the tempo of the angiosperm radiation and concurrent changes in the systematic diversity of other groups of terrestrial plants. We compiled data from 197 floras of Late Jurassic, Cretaceous and Palaeocene age, tabulating the occurrence of over 3,500 macrofossil species. We then assessed changes in the diversity of angiosperms, conifers, pteridophytes (ferns, lycopods and sphenopsids), cycadophytes (cycads, Bennettitales and pteridosperms with simple pinnate leaves), ginkgophytes (Ginkgoales and Czekanowskiales) and other seed plants (Caytoniales and other taxa). Our analysis is based solely on the fossil leaves of these six plant groups because they can be readily distinguished and together comprise over 88% of all species recorded in the floras examined. In the absence of a standardized systematic treatment of Cretaceous floras we did not attempt to synonymize species citations.

Analysis of absolute species diversity ('species richness', Fig. 1) shows only a moderate increase in the total diversity of terrestrial plants after the mid-Cretaceous, despite a major and geologically rapid rise in the number of angiosperm species. Conifer diversity shows a slight decline, but the number of pteridophyte species falls sharply and the previously important cycadophytes are almost totally eliminated. Analysis at the generic level (Fig. 2) shows similar, though not identical, patterns.

Quantitative analyses of the palaeontological record⁶⁻¹² underestimate actual diversity^{13,14} and are susceptible to a range of sampling biases^{8,14,15}. To minimize these problems, to test the uniformity of trends in absolute diversity per interval, and to reduce effects of unequal sampling, we analysed the percentage contribution of angiosperms, cycadophytes, pteridophytes and conifers to individual fossil floras through time (Fig. 3). Within-flora diversities show clear trends consistent with results of earlier studies^{11,12} and our analyses of absolute species and generic diversity. Within-flora percentages of angiosperm species increase rapidly between about 115 to 90 million years BP, rising from near zero to a mean of ~70%, with virtually no Late Cretaceous floras having less than 30% angiosperms. Representation of conifers remains more or less constant despite significant systematic changes, notably the decline of the Cheirolepidiaceae¹⁶. Cycadophytes and pteridophytes show marked reduction in diversity, such that they rarely account for more than 30% of species in Late Cretaceous floras. To moderate

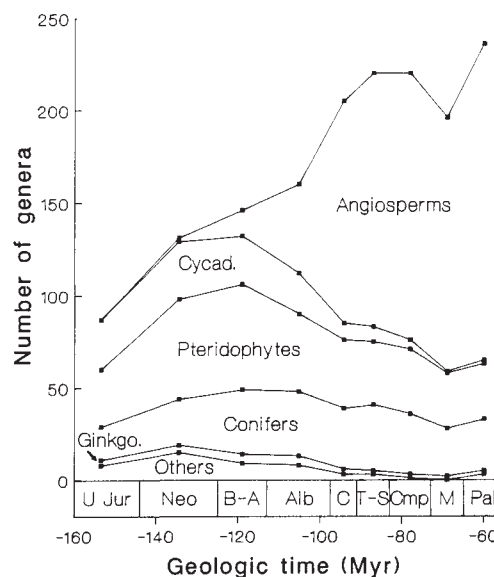


Fig. 1 Absolute systematic diversity ('richness') of tracheophytes reveals a striking increase in angiosperm taxa during the Albian and Cenomanian, and approximately concurrent changes among other major groups. Species richness is summed from 197 Late Jurassic to Palaeocene macrofossil floras (list available on request). Each flora was assigned an age equivalent to the midpoint of its possible time-stratigraphic range, derived from recent stratigraphic estimates, and was placed in one of nine major time intervals for diversity calculations. Plant fossils *incertae sedis* and taxa based on reproductive organs, wood, roots or vegetative remains of other groups (for example algae and bryophytes) were not considered. The timescale employed is that of Harland *et al.*³³. Uneven temporal distribution of floras and fifteenfold differences in stage durations during the time period considered present significant *a priori* biases for direct comparisons of 'summed' diversities among stages. We therefore combined occurrences in the Late Jurassic, Neocomian, Barremian-Aptian and Turonian-Santonian to attain more uniform 'interval' lengths and numbers of floras sampled per 'interval', and to incorporate floras whose stratigraphic resolution overlaps stage boundaries. The flora of the Dakota Sandstone is excluded from these and subsequent analyses as being obviously anomalous. The 432 vascular plant species reported by Lesquereux³⁴ equal 85% of our total Cenomanian diversity, and at the species level the flora is nearly 70% larger than any other flora considered. Note that this exclusion biases against, not for, our principal conclusions. The Dakota flora is dominated floristically by flowering plants and would further elevate the number of angiosperm species originating during the mid-Cretaceous interval. Cycad., cycadophytes; Ginkgo., ginkgophytes.

potential effects of inconsistent systematic treatments further, the within-flora analysis was repeated on a sequence of 24 fossil floras described by a single worker¹⁷⁻²⁰, and revealed the same major trends. The macrofossil record thus clearly shows a rapid radiation that established angiosperms as the dominant group in terms of species diversity throughout the Northern Hemisphere as early as the Cenomanian. Although this conflicts with most previous quantitative analyses of the macrofossil record⁶⁻⁹ it compares favourably with palynological data which suggest that angiosperms first became floristically dominant during the Turonian^{4,5}.

Previous assessments of absolute species diversity in the plant fossil record have registered an initially slow angiosperm diversification accompanied by a gradual decline among contemporary Mesozoic plants⁶⁻¹⁰, and this has been used to support the view that the mid-Cretaceous radiation of angiosperms resulted in competitive exclusion of previously important elements in the Mesozoic vegetation^{6,7}. Although palaeontological patterns are rarely more than suggestive of competitive displacement²¹, the pronounced trends in our data could be interpreted

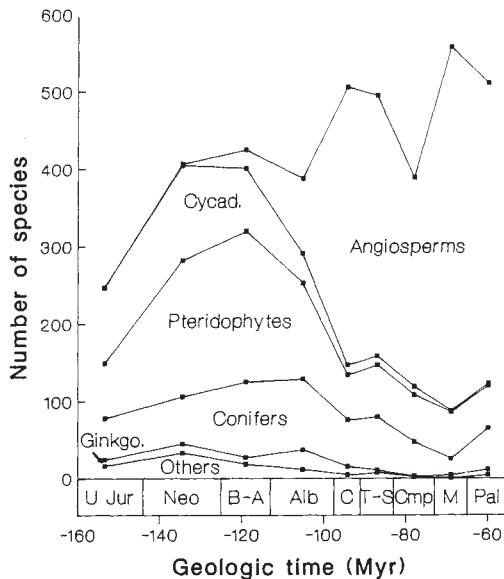
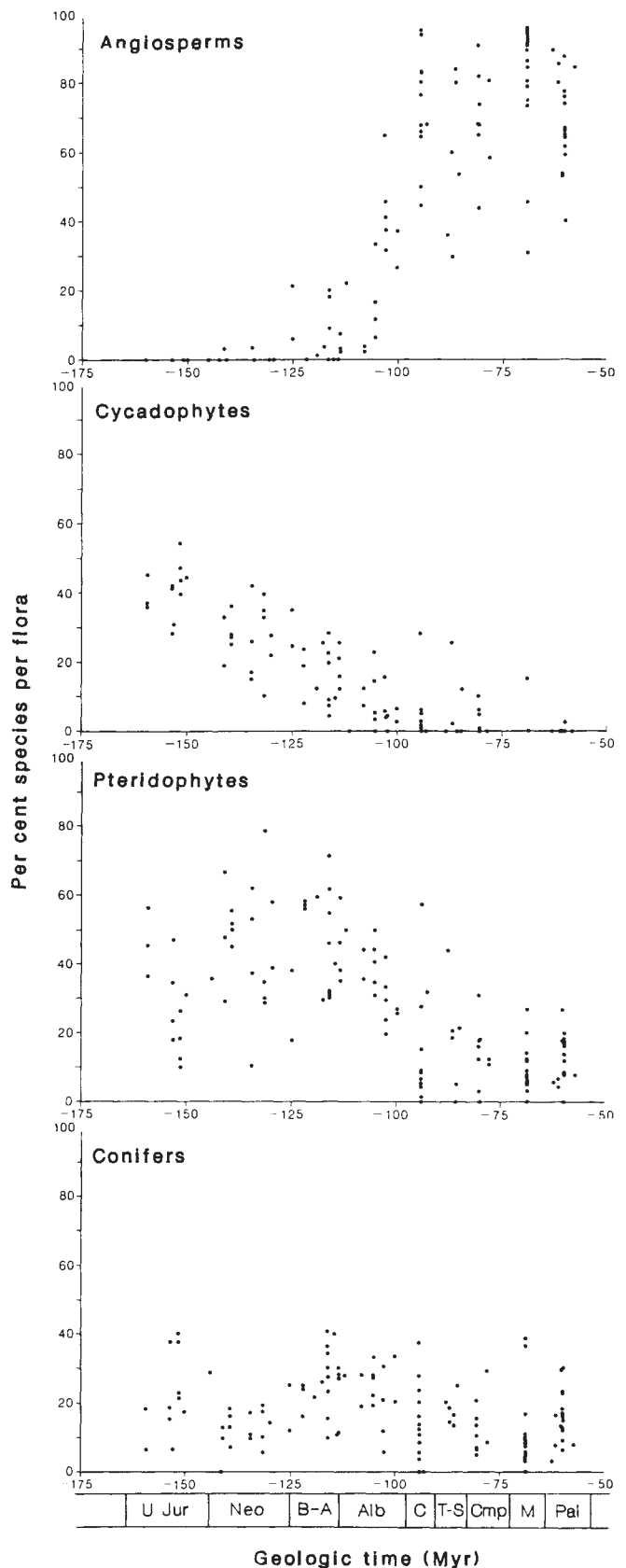


Fig. 2 Total genus richness, calculated as in Fig. 1 and over the same time period. The overall pattern and tempo of change among major groups is similar to that at the species level, but shows less fluctuation. This is consistent with a tendency for diversity analyses at supra-specific levels to be less susceptible to sample error than those at the species level³¹, and suggests that whereas improved systematic and stratigraphic information may enhance the resolution of these data, the fundamental patterns in proportions of major taxa within intervals should not be altered substantially.

Fig. 3 Percentage contribution of major taxa to individual Late Jurassic through Paleocene floras. Unlike absolute compilations of species richness, within-flora comparisons require only that species of leaf macrofossils from single floras are distinct and correctly assigned to a major group. Because proportions of major groups in any one flora must sum to 100%, the power of these analyses depends on the consistency with which many floras independently conform to an overall pattern through time. Angiosperm species are the dominant element in virtually all macrofossil floras following a geologically rapid floristic transition in the mid-Cretaceous. The continued dominance of angiosperms is consistent with previous summaries of floral composition^{11,12} which estimated that Late Cretaceous floras as a whole typically contain 65–70% angiosperms. Within-flora percentages of cycadophyte and pteridophyte species decrease significantly through the mid-Cretaceous, whereas conifers show little or no relative decline. Percentages are derived from 131 floras with 10 or more leaf macrofossil species (mean = 38 species per flora), in order to lessen the potential effects of small sample sizes and patterns of occurrence of rare species.

as supporting such a hypothesis, implying that cycadophytes and pteridophytes rather than conifers were most affected by angiosperm competition. Conifer diversity shows little quantitative change between the Late Jurassic and Palaeocene, whereas pteridophytes show a clear decline during the rapid angiosperm diversification, followed by a more gradual decline in the Late Cretaceous and early Tertiary. Curiously, cycadophytes show a marked decline in their percent contribution to fossil floras before as well as during the rapid phase of angiosperm diversification. This is not clearly reflected in a decrease in absolute cycadophyte diversity (Figs 1 and 2), and results from fluctuations in the relative importance of pteridophytes and conifers in floras prior to ~120 Myr BP. The decline in the importance of cycadophytes in pre-angiosperm floras implies that complex changes in floristic composition may have been initiated before the major angiosperm radiation, and could have accelerated the diversification of angiosperms, and perhaps other groups that radiated during this interval (for example Gnetales^{5,22}). Thus alternative hypotheses involving climatic perturbations²³,



biogeographic effects², declines in non-angiosperm taxa and differential contributions from sub-clades within early angiosperms²⁴⁻²⁹ need to be evaluated in assessing possible competitive effects associated with the angiosperm radiation.

When angiosperms are considered in isolation from other groups, the absolute diversity patterns (Figs 1 and 2), within-flora diversities (Fig. 3), and taxonomically standardized subsets of within-flora diversities show a similar sigmoidal pattern consisting of a low diversity phase, followed by rapid diversification, followed in turn by a period of fluctuation about a more or less stable plateau. With the exception of two species, angiosperm macrofossils recorded before ~120 Myr BP (Fig. 3) are based on fragments of supposed monocotyledonous leaves which cannot be unequivocally determined as angiospermous. Thus, although angiosperms certainly existed in the Barremian, Hauterivian and perhaps earlier³⁰, they are very poorly represented as macrofossils. During the early phase of radiation measured here, and especially during the peak period of diversification, angiosperms exhibit total rates of generic origination³¹ as much as four times greater than those recorded over a similar time period during radiation of early vascular plants³². Notwithstanding the inherent difficulties of such direct comparisons^{8,15,31}, the diversification of angiosperms clearly occurred at an extraordinary rate which is also reflected in considerable phylogenetic differentiation within the group. Plant fossils closely resembling several extant families (Magnoliaceae²⁴; Winteraceae²⁵; Platanaceae²⁶ and Chloranthaceae^{27,28}) and representing at least four of the eleven extant angiosperm subclasses (Magnoliidae, Hamamelidae, Rosidae and at least one subclass of Liliatae^{1-3,24-29}) appear in the fossil record for the first time before 90 Myr BP.

The pattern of the early angiosperm diversification parallels that reported for marine invertebrates³¹ and early vascular plants³² in approximating a logistic (kinetic) growth model. Although it is uncertain whether the early angiosperm radiation conforms to other predictions of this model³¹, the late Cretaceous plateau in angiosperm diversity contrasts markedly with subsequent pronounced diversification during the Tertiary^{4,5,7-10}. The evolutionary dynamics associated with this renewed angiosperm radiation remain to be determined.

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Local resource enhancement and sex ratios in a primitively social bee

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'Queen-worker conflict'¹ and 'local mate competition'^{2,3} have frequently been used to help explain female-biased sex ratios in the social Hymenoptera. In this report I show that neither of these theories is able to explain brood-size dependent sex ratios in an allodapine bee, *Exoneura bicolor*. Most nests of this species contain two or more related females, and reproductivity per female increases as a function of the number of individuals cooperating in joint nest use. I argue that 'local resource enhancement' may explain sex-ratio bias in this species and that similar bias may be expected in other presocial insects.

The primitively social allodapines^{4,5} are unique among the bees in rearing their brood progressively in an unsealed communal burrow. Because brood are not enclosed within cells, they are dependent on the continued presence of adults for protection from parasites and predators. This, together with progressive rearing, not only places a premium on female longevity, but creates potential for significant fitness enhancement accruing from cooperative brood care.

The Australian allodapine, *E. bicolor*, is univoltine with no overlap of generations during brood-rearing. Two types of colonies, differing in their grade of social organization, have been distinguished⁶: groups of up to 9 females ($\bar{x}=3.8$) may remain permanently in their natal, overwintered nests which they lengthen and re-use for brood rearing. A variable number of females in these nests remain non-reproductive and the resultant colonies may be classed as semisocial. In late spring, other females disperse to initiate new colonies consisting of 1-8 cofoundresses ($\bar{x}=2.7$). There is no apparent reproductive differentiation among cofoundresses, and a single communal clutch of eggs is produced shortly after nest initiation. Newly founded colonies may therefore be classed as quasi-social. Intra-colony relatedness is high in both overwintered (0.485 ± 0.060 , $b \pm s.e.m.$) and newly cofounded (0.597 ± 0.062) colonies⁷, indicating that kin group integrity is maintained during foundress dispersal.

Sex-ratio data (Table 1) were obtained from a sample of 225 nests collected at the end of the brood-rearing season. Both overwintered (OWN) and newly founded (NFN) nests exhibit strongly female-biased sex ratios, with the degree of bias varying inversely with brood size. With each brood-size category, newly founded colonies exhibit greater female bias than colonies in overwintered nests. Although mean male wet-pupal weight exceeds that of females by ~6.5% (ref. 8), modification of parental-investment (PI) ratios from sex ratios in line with this difference does not greatly decrease the degree of bias, particularly in colonies with very few or no males. In eusocial species, queen investment in workers is ultimately directed towards the production of both gynes and males, so that production of workers cannot be counted as PI in females *per se*. Colonies of *E. bicolor* are not eusocial, however, and worker sterility is facultative⁹. Hence the existence of non-reproductive females in some colonies only serves to increase PI in the

remaining reproductive females, so that the proportion of workers cannot be discounted when calculating relative investment from sex ratio data.

Using inclusive fitness arguments, Trivers and Hare¹ predicted that workers in monogynous eusocial hymenopteran colonies are expected to bias PI in reproductives to the ratio 3:1 F:M. Such queen-worker conflict over preferred PI ratios may arise in haplo-diploid species when reproductives are reared by female (worker) siblings. But in *E. bicolor* brood-rearing colonies are composed exclusively of females from a single generation, so that workers are related equally to male and female brood.

Local mate competition (LMC) has also received attention as a possible source of sex-ratio bias in social insects^{3,10}. LMC may cause female-biased PI ratios if breeding structure leads to competition between male kin for matings. Several lines of evidence suggest that LMC is unable to account for the pattern of PI bias observed in *E. bicolor*. Inbreeding has been estimated, using allozyme data, from an exhaustive sample of nests from 20 nesting aggregations⁷, and is not significantly different from zero in either newly founded ($-0.0058 \pm 0.0664 F \pm \text{s.e.m.}$, $c = 98$, $n = 299$) or overwintered (-0.0488 ± 0.0556 , $c = 88$, $n = 369$) colonies. Occurrence of nests in dense aggregations⁶, lack of significant within-aggregation relatedness⁷, and an extended mating period (March to December⁶) make the existence unlikely of mating arenas that result in competition between male kin but prevent inbreeding. More importantly, the proportion of males under LMC is expected to be most pronounced, or even total, in the smallest broods, with increasing female bias in larger broods^{11,12}. The reverse pattern of investment is found in *E. bicolor*, however, with extremely low proportions of males in very small broods and decreasing bias in larger broods (Table 1).

Clark¹³ suggests that male-biased PI ratios in a prosimian primate may be due to 'local resource competition' (LRC) among female siblings, such that the mean fitness of daughters

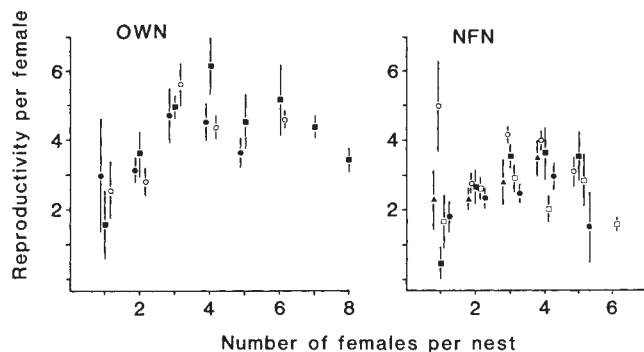


Fig. 1 Mean reproductivity per female¹⁵ as a function of the number of females per nest in overwintered (OWN) and newly founded nests (NFN) of *E. bicolor*. Each point represents the arithmetic mean number of brood per female calculated over all nests for each colony size. Bars indicate $\pm$ mean standard error. Means are provided only for colony sample sizes greater than 1. Nests were collected during periods of rain or before sunrise when all adult colony members could be assumed present. Nests containing the cuckoo bee *Inquilina* are excluded from analyses. Sample dates are as follows: $\square$, 30 November, 1983; $\blacksquare$, 30 November–4 December 1985; $\circ$, 1–5 January 1984; $\bullet$, 15–31 January 1985; $\blacktriangle$, 24 January 1984.

provides a cross-sectional measure of reproductive interaction and does not account for colony failure before or after sampling. Consistently low frequencies of single female nests throughout brood rearing⁶, however, suggest that colony survivorship is greater for multi-female nests. Ants maintain a significant presence around *Exoneura* nest entrances⁶ and are likely to pose a major predatory threat. It is probable that defence from predators and parasites is more effective in larger colonies with an increased adult presence in an unbranched nest with one entrance. Additional benefits at both individual and colony levels may also be expected from division of labour in multi-female nests.

The relationship between reproductivity per female and colony size in *E. bicolor*, together with kin association of cofoundresses, suggest that the mean fitness of female offspring increases with the number of females in a brood. If clutch size is limited by the rearing capacity of females, then the number of females in a brood will be a function of the sex ratio, and selection will favour female-biased PI. As the number of females within a brood increases, a point will be reached when production of additional females no longer results in a further increase in mean daughter fitness. Such a limit to fitness enhancement is suggested by the apparent decrease in mean reproductivity per female when colony size exceeds 4–5 females per nest. Hence bias is expected to be less pronounced in large broods. Such a trend is apparent in Table 1. Because kin integrity is maintained during cofounding, bias is expected even if all females ultimately disperse from their natal nests. However, different rates of nest re-use and foundress dispersal by females raised in newly founded and overwintered nests⁷ suggest that optimal PI ratios will be different for these two colony types.

Cooperative nest use by female kin, maintained by mutualistic benefits, is postulated to be an early step in insect social evolution¹⁶. Although *E. bicolor* is univoltine, LRE could also occur in bivoltine species with overlapping generations. The effect of female-biased sex ratios in lowering threshold conditions for eusociality in haplo-diploid species¹⁷ suggests that LRE may have played a facilitative role in hymenopteran social evolution.

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Table 1 Sex-ratio data from 225 overwintered (OWN) and newly founded (NFN) nests of *E. bicolor* collected at the end of the brood rearing season (February 1987)

Brood size	Nest type	Number of nests		Total pupae		Male proportion of brood
		(total)	with males	Female	Male	
1–3	NFN	29	2	61	3	0.047
	OWN	16	3	30	4	0.125
4–6	NFN	45	3	217	3	0.014
	OWN	14	6	65	10	0.133
7–9	NFN	36	9	270	10	0.036
	OWN	16	10	96	30	0.238
10–15	NFN	24	13	237	42	0.151
	OWN	15	12	138	44	0.242
>15	NFN	18	14	265	69	0.207
	OWN	13	12	176	87	0.331

Nests were only used if all immatures present could be sexed. Prepupae, when present, were maintained at room temperature until pupal eclosion. Nests containing feeding larvae or immatures which were diseased or parasitized, or nests containing the cuckoo bee *Inquilina* were excluded from analyses. Male proportion of brood is calculated from brood pooled for each brood size category.

decreases with the number of females in a brood. Clark also notes that within-sex interactions can theoretically be positive, leading to 'local resource enhancement' (LRE). Positive fitness interactions between kin have recently been suggested as a source of sex-ratio bias in birds with helpers at the nest¹⁴.

In *E. bicolor*, the high proportion of multi-female nests during brood rearing suggests that joint-nest use confers significant fitness benefits to cooperating females. Much of the information on cofounding^{6,7} comes from aggregations which have been saturated with trap-nests, indicating that cofounding is not a response to limited nesting sites. Female reproductive efficiency is compared between different-sized colonies, using estimates of mean reproductivity per female¹⁵ (Fig. 1). There is an overall trend of increased reproductivity per female concomitant with increasing colony size up to 5 females per nest. This parameter

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Absence of interhemispheric connections of area 17 during development in the monkey

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Our understanding of the development of cortical connectivity largely stems from studies of the ontogeny of interhemispheric pathways in carnivores, rodents and lagomorphs¹⁻¹². Early in development, cortical neurons projecting to the contralateral hemisphere through the corpus callosum (callosal projection neurons) have a widespread distribution. As maturation proceeds, callosal projection neurons become restricted to those cortical regions that are connected in the adult. In newborn cats and rats, for example, callosal projection neurons are not restricted to the 17-18 border as in the adult, but are found throughout areas 17 and 18 (refs 2, 5 and 10). The macaque monkey is an exception, because at birth it has an adult-like distribution of callosal projection neurons in area 18, with practically none in area 17 (refs 13-15). Here we show that whereas area 17 is devoid of interhemispheric connections throughout prenatal development, the distribution of callosal projection neurons in area 18 shows the common sequence of an early widespread distribution followed by regression. The absence of callosal projection neurons in area 17 throughout ontogeny may well be a feature unique to Old World primates.

We have investigated the callosal connectivity of areas 17 and 18 using horseradish peroxidase (HRP) histochemistry in fetal macaque monkeys (*Macaca cynomolgus*) at 97 (±2), 112 (±2), 114 (±2) and 133 (±2) days after conception (gestation period is 165 days) (Fig. 1).

At a fetal age of 97 days (E97), anterogradely labelled callosal fibres had only just crossed the midline, and no retrogradely labelled neurons were found in the hemisphere contralateral to the injection. The lateral geniculate and pulvinar nuclei ipsilateral to the injected hemisphere were extensively labelled both retrogradely and anterogradely, indicating that pick-up and transport of HRP had occurred. By 114 days (E114), labelled neurons were found in the contralateral hemisphere. At this age the cytoarchitectonics of area 17 are quite distinctive, and its border with area 18 is as sharply defined as in the adult. Callosal projection neurons were found at the 17-18 border, but nowhere else in area 17. We have checked that HRP was taken up in the injected striate cortex by examining the ipsilateral lateral geniculate nucleus, which contained numerous labelled neurons. A dense band of labelled callosal projection neurons was found

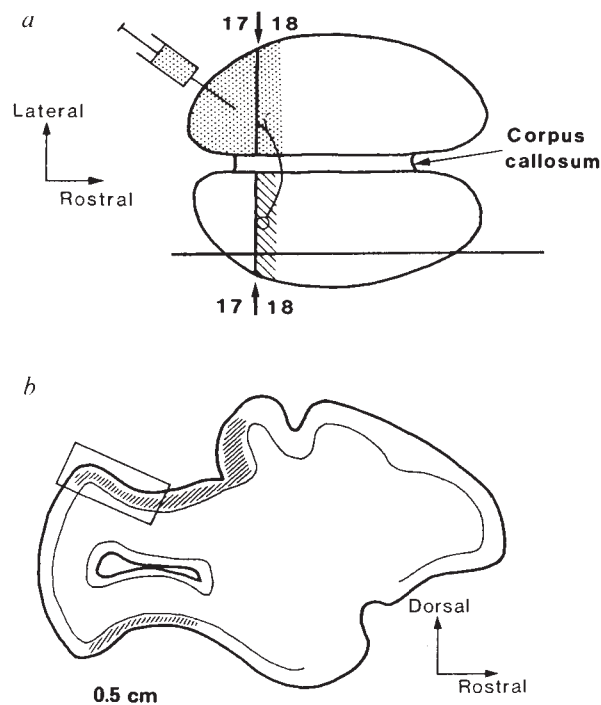


Fig. 1 *a*, The experimental paradigm. HRP is injected at multiple sites in the occipital lobe of one hemisphere so as to fill massively area 17 and the adjacent part of area 18 on the dorsal cortical surface. HRP is captured in the injected hemisphere and transported retrogradely to cell bodies in the contralateral hemisphere. After a suitable survival period to allow axonal transport, the contralateral hemisphere is examined for HRP reaction product. *b*, A parasagittal section (taken along horizontal line in *a*) of a prenatal monkey brain at embryonic day E114. At this age the lunate sulcus is not yet formed. Retrograde labelling of neurons is found in both the ventral and dorsal cortical surfaces, showing that at this age the interhemispheric pathway is in place. In this and all other embryonic ages examined, retrogradely labelled cells in the contralateral hemisphere were found extensively in area 18, but stopped abruptly at the 17-18 border. Square on dorsal surface shows location of high-power plot presented in Fig. 2.

Methods. Timed pregnant monkeys were prepared for surgery under Alfatesine (IV) anaesthesia. After intubation, anaesthesia was continued with halothane in N₂O/O₂ (70:30) mixture. Expired CO₂ and heart rate were monitored. After a midline abdominal incision, uterotomy was performed and the fetal head exposed. Craniotomy was performed over the occipital lobe and 3-5 µl of HRP (30-50% solution) were injected by Hamilton syringe so as to fill massively areas 17 and 18 on the dorsal cortical surface. Injections (5-7) were made at a shallow angle so that each injection spanned 4-6 mm of cortex. All incisions were closed using routine procedures and the mother medicated with a muscular relaxant (Duvadilan) and analgesic (Visceralgine) before being returned to her cage. The fetus was delivered by caesarian section 36-48 h later, deeply anaesthetized and perfused through the heart with a mixture of 1.25% paraformaldehyde and 1.5% glutaraldehyde. Routine procedures were used for HRP histochemistry using tetramethylbenzidine (TMB) as the chromogen¹⁶.

in anterior cortical regions stretching posteriorly throughout area 18, but terminating abruptly at the 17-18 border (Figs 2 and 3). Area 18 on the ventral surface of the hemisphere also contained large numbers of labelled neurons (Fig. 1*b*). All labelled neurons in this and the other animals were found in supragranular layers. Although, as often found in the neonate¹⁴, the density of HRP reaction product was relatively low in the cells, use of crossed polarized filters made it possible to visualize labelled cells and to chart their position using an x-y plotter electronically coupled to the microscope stage. This showed that densities of labelled cells were very high in extrastriate cortex

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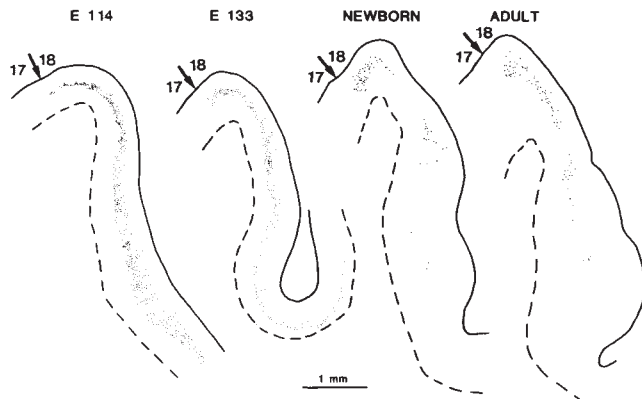


Fig. 2 Parasagittal 60 µm-thick sections of the area 17-18 border of fetal (E114, E133), newborn and adult monkeys. The arrow on the left of each brain section indicates the 17-18 border determined after counterstaining for Nissl substance. The dotted line indicates the boundary between grey and white matter. Each dot represents a retrogradely labelled neuron, following injection of HRP in the contralateral areas 17 and 18. In adult visual cortex, callosal neurons are largely confined to area 18 where they occur in clusters. The same distribution is observed in the newborn monkey. At E133, the distribution of callosal neurons in area 18 is continuous and no periodic fluctuation of the density of labelled neurons is observed. At E114, the density of labelled neurons appears considerably higher than at later ages and there is no suggestion of a periodic distribution. At all prenatal ages investigated, very few callosal projection neurons were found in area 17 where they were located on the 17-18 border. In all cases, callosal projection neurons were located in supragranular layers. For orientation of plot, see Fig. 1b.

and that there was no obvious periodic fluctuation of numbers of labelled neurons. The same results were obtained at embryonic day E112.

Callosal connectivity was examined in one animal at day E133. Again, no callosal projection neurons were found in area 17, despite the fact that the lateral geniculate nucleus contained numerous retrogradely labelled neurons. At day E133, the plots of retrogradely labelled neurons in extrastriate cortex showed an overall drop in density, compared with E112 and E114 (Fig. 2). The periodic spatial fluctuation of the density of callosal projection neurons in area 18, characteristic of the newborn and adult, was not present at E133 (Fig. 2).

Our data demonstrate that the callosal projection from area 18 completes its maturation between E133 and birth. This time course is similar, although delayed, to that of the development of callosal connections in the somatosensory cortex of the macaque monkey which begins around E119 and is completed by E133 (ref. 19). This delay in the reorganization of callosal connections of area 18, compared with somatosensory cortex, possibly reflects known rostro-caudal maturation gradients in the developing nervous system.

These results indicate that area 17 does not send axons to the contralateral hemisphere at any time in development in the macaque monkey. It is possible that callosal projection neurons are present in area 17, but for a much shorter period than that in which callosal projection neurons are undergoing reorganization in area 18. It seems unlikely that there are callosal projection neurons in area 17 before day E112. We found at E97 that callosal axons still had about 1-1.5 cm to grow before reaching the cortical grey matter of the contralateral hemisphere. Rates of axon growth range from 1.25 to 2.6 mm per day^{17,18} so that one could expect this pathway to be complete by about E107, leaving only a five-day period unexplored in this study. The possibility remains that callosal projection neurons are present in area 17 for only a brief period either before or after E133. In either case, both the onset and duration of the maturation of area 17 callosal connections would have to be very different

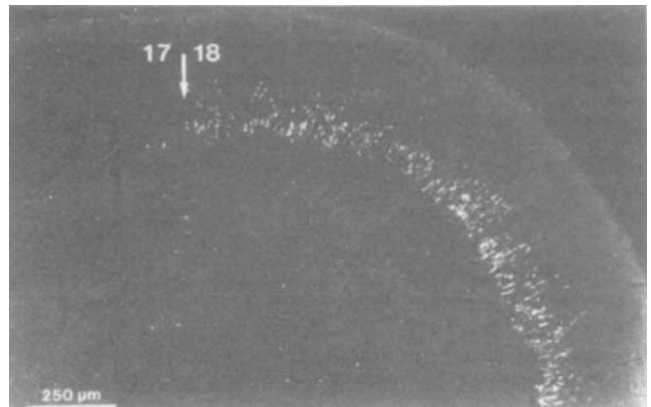


Fig. 3 Darkfield microphotograph of retrogradely labelled neurons from the section shown in Fig. 1b of the E114 fetal monkey. The white arrow indicates the 17-18 border. Callosal neurons are densely packed and lie in superficial layers. Scale bar: 250 µm.

from that found in other cortical areas, such as area 18 or the somatosensory cortex¹⁹.

The absence of callosal projection neurons in area 17 during ontogeny in the macaque is exceptional compared with other mammalian species. This raises the question of whether this is a general primate feature. There are no reports on the development of callosal connections of New World and prosimian primates. It is known, however, that numerous callosal projection neurons are found within area 17 in the adults of some species of these two suborders, and it is possible that these neurons constitute the remnants of an earlier extensive population of callosal projection neurons²⁰. The absence of callosal projection neurons in area 17 during development might therefore not be a common feature of all primates, but instead could be specific to Old World primates.

Studies both on inter-¹⁻¹² and intrahemispheric²¹⁻²⁵ connections has led to the commonly accepted notion that cortical connections are more widespread during early development and that maturation is accompanied by a restriction of this connectivity. The present results suggest that this pruning of connections is not the only mechanism involved in the establishment of cortical connectivity. We cannot exclude the possibility that area 17 neurons send axons towards the corpus callosum, but over a very limited time so that they do not reach the contralateral hemisphere. Alternatively, the degree of specification of the connectivity of area 17 neurons might be much higher than for other cortical areas in the monkey, including the primary somatosensory cortex which does show exuberant callosal connections¹⁹. Should this be the case, area 17 of Old World monkeys might be expected also to be free of other transient connections which characterize the maturation of visual cortex in other species. These connections include transient projection to the pyramidal tract in carnivores²⁶, rodents^{27,28}, and lagomorphs¹⁷ and the projection of auditory cortex to area 17 in carnivores²²⁻²⁵.

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Segregation of functionally distinct axons in the monkey's optic tract

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The classical neuro-ophthalmologic literature describes the organization of the primate's optic tract as containing a single topographic representation of the complete contralateral visual hemifield^{1–4}. In contrast, cats have separate visual field representations for the optic axons of the functionally distinct retinal ganglion cell classes^{5,6}. As the line of decussation for each ganglion cell class in the cat occupies a different location on the retinal surface^{7–11}, whereas in primates they are all superimposed¹², such a species difference might be expected. We report that implants of horseradish peroxidase placed in either the deep or superficial extremes of the monkey's optic tract produce retrograde labelling of distinct retinal ganglion cell classes, and produce anterograde labelling confined to distinct laminae of the lateral geniculate nucleus. Hence, the optic tract of the primate cannot contain a single representation of the contralateral visual hemifield; rather, independent visual field representations for the functionally distinct optic axons must exist. Their anatomical segregation may account for the clinical observation of selective impairments of distinct visual abilities following partial interruption of the optic tract in man¹³.

Implants of dried gel-foam that had been previously saturated with horseradish peroxidase (HRP) were surgically inserted into one optic tract of five old-world monkeys, at varying deep-to-superficial locations (that is, removed from or near to the outer, pial, surface) within the optic tract. Such implants retrogradely labelled retinal ganglion cells across large areas of the two respective half-retinae of the contralateral and ipsilateral eyes in all five cases. Implants at more medial locations in the optic tract produced retrograde labelling of cells in more dorsal retinal locations, confirming the classical description of the superior-inferior retinal topography within the tract^{1–4}. But the deep-to-superficial axis of the tract did not correspond to any simple foveo-peripheral retinal topography, as demanded by these descriptions. Rather, labelled ganglion cells were found across most retinal eccentricities. Implants of HRP at different depths in the tract produced a striking difference in the relative proportions of the morphological cell classes that were retrogradely labelled. Figure 1 displays two extreme cases, with implants at deep and superficial (sub-pial) positions within the tract (Fig. 1a, b). The former retrogradely labelled a population of retinal ganglion cells with very small somas and tightly restricted dendritic arborizations, characteristic of the primate's P_β cell^{14–17} (Fig. 1c). Anterograde labelling in the lateral geniculate nucleus (LGN) in this case was restricted to its lateral aspect, primarily in the parvocellular laminae, with no labelling in magnocellular lamina 1 (Fig. 1e). The superficial (sub-pial) implant produced

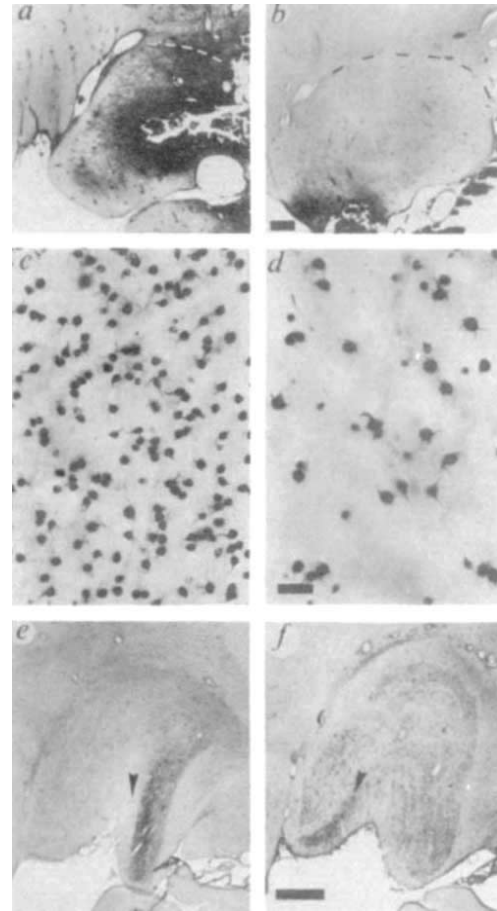


Fig. 1 a, b, Coronal sections through the optic tract of two monkeys receiving implants of HRP into the deep or superficial (sub-pial) regions of the optic tract, respectively. The deep border of the optic tract is indicated by the broken line. Dorsal is up, medial is to the left. Calibration bar = 500 μ m. c, d, Retrogradely labelled retinal ganglion cells at an eccentricity of 5 mm in the nasal (contralateral) retina following an HRP implant into the deep or superficial optic tract, respectively. Calibration bar = 50 μ m. e, f, Anterograde labelling of optic axons and terminals in the parvocellular or magnocellular laminae of the LGN following implants of HRP into the deep or superficial optic tract, respectively. Arrowhead in e indicates absence of labelling in magnocellular lamina 1 of the LGN, whereas arrowhead in f indicates its prominence there. Dorsal is up, medial is to the left. Calibration bar = 1 mm.

Methods. Implants of HRP were surgically inserted into one optic tract under visual control. The surgical approach, carried out under strict aseptic precautions while the monkey was deeply anaesthetized, was through the temporal lobe. Overlying tissue was aspirated to reveal the optic tract's lateral surface, at a position approximately midway between the optic chiasm and the lateral geniculate nucleus. A small hole was made in the tract with fine forceps, damaging a local region of optic axons, into which the HRP was then inserted. Monkeys were perfused 48–72 h later with 0.9% saline followed by 2% paraformaldehyde in 0.1 M phosphate buffer at 4 °C. The eyes were removed immediately, the retinae dissected out and reacted for HRP histochemistry^{28,29}, and retinal whole-mounts were prepared. After the eyes were removed, the perfusion was then continued with 1.25% paraformaldehyde + 2.5% glutaraldehyde in phosphate buffer. Frozen sections were cut coronally at 50 μ m through the brain and reacted for HRP histochemistry using each of two protocols^{28–31}.

retrograde labelling of a population of ganglion cells roughly half of which possessed a substantially larger soma, more primary dendrites and a larger dendritic arborization—characteristics of the primate's P_α cell^{14–17} (Fig. 1d). The anterograde labelling in this case was confined to the medial aspect of the

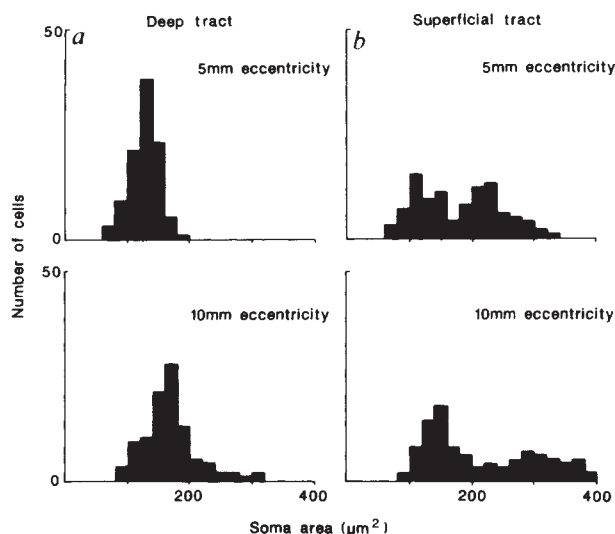


Fig. 2 *a, b*, Soma area distributions at 5 and 10-mm eccentricity following implants of HRP into the deep or superficial (sub-pial) optic tract, respectively. Both distributions shift to larger sizes at the more eccentric retinal position, as expected from the known increase in soma size with eccentricity for any given cell type^{16,18}. Cell densities for each of these sampled regions are: *a*, 5 mm eccentricity = 2,240 cells mm⁻²; 10 mm eccentricity = 232 cells mm⁻²; *b*, 5 mm eccentricity = 278 cells mm⁻²; 10 mm eccentricity = 208 cells mm⁻².

Methods. Profiles of 100 adjacent retrogradely labelled retinal ganglion cells were drawn at a magnification of $\times 1,000$ with the aid of a drawing tube attached to the microscope, and areas of cell somas were measured with a digitizing tablet linked to a microcomputer. Samples were taken at each of two eccentricities (5 and 10 mm). Adjacent regions of retina $100 \mu\text{m} \times 100 \mu\text{m}$ were examined until 100 labelled cells were encountered and drawn. Samples regions progressed from the stated eccentricity towards the fovea. Cell density was also determined at eccentricities from 2 to 15 mm by counting all labelled profiles in regions $200 \mu\text{m} \times 200 \mu\text{m}$ at 1 mm intervals, using a $\times 100$ oil-immersion objective. Both implants yielded a greater number of labelled cells near the fovea than in the periphery: a comparison of density of labelled cells at 2 and 15 mm eccentricity for the two cases indicates a 50-fold decline for the deep implant and a 3-fold decline for the superficial implant.

magnocellular laminae of the LGN, being densest in magnocellular lamina 1 (Fig. 1f).

To quantify the difference in the populations of retrogradely labelled ganglion cells, cell soma sizes were measured at each of two eccentricities (5 and 10 mm) for 100 cells in the contralateral (nasal) retina of these two cases (Fig. 2). A comparison of the respective cell size distributions indicates that distinct sub-populations of the retinal ganglion cell population at each eccentricity were labelled in each case. The distribution for the superficial implant is clearly bimodal, whereas that for the deep implant is unimodal, containing no large somas. Hence, no large ganglion cells at these retinal locations send their axons through deep portions of the optic tract. Yet this population of ganglion cells is readily labelled following implants at superficial positions (Fig. 2b). Many of the smaller ganglion cells labelled following a superficial implant did not show adequate dendritic labelling to permit identification of their morphology; many which did were characteristic of retinal ganglion cells that project to the midbrain¹⁸.

We have previously shown that there is a partial segregation of optic axons according to size in the monkey's optic tract, fine diameter axons being distributed across the tract and coarse diameter axons being found primarily superficially, near the pial surface¹⁹. The present results demonstrate that this segregation by size is in fact a segregation by type, the optic axons

arising from the P _{β} and P _{α} ganglion cells at a given point on the retina coursing through the tract at widely separate locations. As the morphologically and physiologically distinct retinal ganglion cell classes are presumed to underlie characteristically distinct visual abilities²⁰⁻²⁴, this segregation of functionally distinct optic fibres may be the anatomical basis for selective perceptual impairments of form and/or colour versus movement following partial interruption of the optic tract in man¹³. Our description of the organization of the visual pathways in the adult primate may also be of significance to developmental studies. Spatial position within the mammalian optic tract can be regarded as an index of the time at which axons arrive during the development of this fibre pathway: later arriving axons occupy more sub-pial positions, displacing the older optic axons to relatively deeper positions^{5,25-27}. Our results would then lead to the prediction that neurogenesis of the P _{β} retinal ganglion cell class begins substantially earlier than that of the P _{α} cells.

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Transduction in taste receptor cells requires cAMP-dependent protein kinase

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In taste chemoreception, cyclic adenosine monophosphate (cAMP) appears to be one of the intracellular messengers coupling reception of stimulus to the generation of the response. The recent finding that sweet agents cause a GTP-dependent generation of cAMP¹ poses the question of how this cytosolic messenger acts at the membrane of taste receptor cells. We have shown that cAMP causes a substantial depolarization in these cells². Here we show with whole-cell recordings and inside-out membrane patches that the depolarization caused by cAMP is accounted for by the action of cAMP-dependent protein kinase, which inactivates potassium channels predominantly of 44 pS conductance. Thus, intracellular signalling of the gustatory cells differs from that of olfactory³ and photoreceptor cells^{4,5}, where cyclic nucleotides control

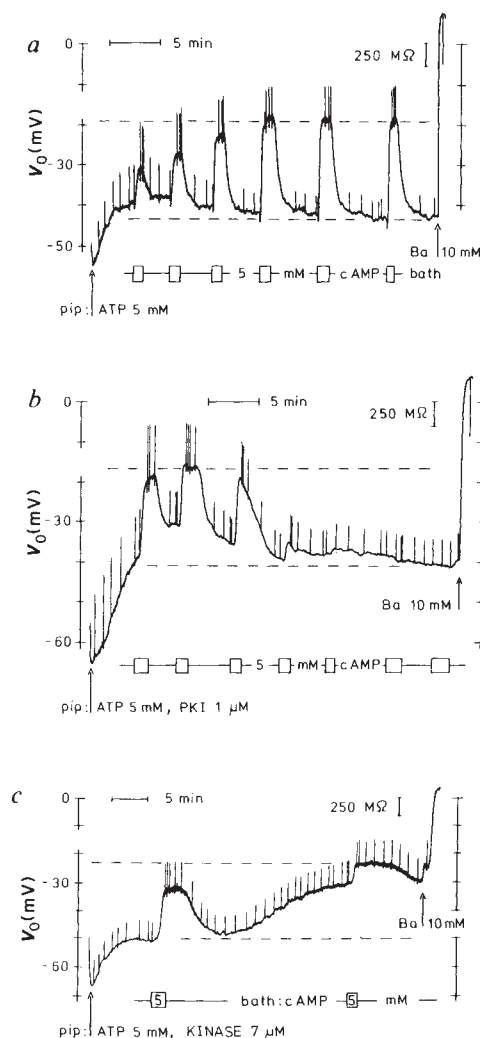
Fig. 1 Voltage records obtained from frog taste-receptor cells in the whole-cell patch configuration during current clamp. *a*, Control; *b*, with protein kinase inhibitor ($1 \mu\text{M}$); *c*, with purified catalytic subunit of cAMP kinase ($7 \mu\text{M}$). Records begin at the time of patch breakage. The zero-current membrane potential V_0 (continuous traces) was interrupted by brief depolarizing current pulses of 26 pA to measure membrane resistance (calibration bars). The dashed horizontal lines delimit the regulatory voltage range of cAMP. Bath solution (mM): NaCl, 110; KCl, 1; CaCl_2 , 1; MgCl_2 , 0.5; HEPES/Tris (pH 7.3), 10; without or with 5 mM cAMP as indicated. Patch-pipette filling solution (mM): KCl, 110; MgCl_2 , 2; EGTA, 0.1; HEPES/KOH (pH 7.2), 10; Na_2ATP , 5. The time required (means $\pm$ s.d.) for full suppression of the depolarizing effect of cAMP was $28 \pm 10 \text{ min}$ after breakage of the patch in six whole-cell experiments with PKI. In four of five whole-cell experiments with the cAMP-kinase the enzyme caused a depolarization from $-46.5 \pm 3 \text{ mV}$ to $-32.2 \pm 2 \text{ mV}$ measured $34.5 \pm 7 \text{ min}$ after breakage of the patch. At this time the remaining depolarizing effect of superfusion with 5 mM cAMP was only $3.1 \pm 2.4 \text{ mV}$ (compared with $26 \pm 6 \text{ mV}$ in 12 control cells).

Methods. Frogs (*R. esculenta*) were decapitated and pithed, the tongues removed and 100–200 taste buds per tongue clipped off under the dissection microscope. A low Ca/collagenase/mechanical agitation treatment dissociated the taste buds into single cells². Sensory cells, recognized by their typical bipolar shape², attached to the glass bottom of the chamber and were patch-clamped⁶ on dendrite or soma under microscopic control. The bath volume ($100 \mu\text{l}$) in the chamber was exchanged by slow perfusion (gravity feed and suction, 12 bath volumes per min), to change extracellular concentrations (whole-cell recordings) or concentrations at the cytosolic face of inside-out excised membrane patches. PKI and the catalytic subunit of cAMP-kinase were prepared by established procedures^{9,11}. PKI was 90% and cAMP-kinase 100% pure by SDS-gel electrophoresis. PKI had a specific activity¹⁰ of 43,000 units per mg protein. The kinase transferred $40 \mu\text{mol}$ phosphate min^{-1} per mg enzyme (4×10^7 units mg^{-1}) to Kemptide (phosphate acceptor peptide). The enzyme was dialysed for 60 h against appropriate high- K^+ salt solutions before use. As these proteins interfered with gigaseal formation, a dilution of their dialysed stock solution was backloaded into the pipette for whole-cell recording, such that the first $100 \mu\text{m}$ of the tip were initially protein-free. The backloading will mainly be responsible for the pronounced diffusion delay (M. Pusch and E. Neher, personal communication) in the action of these agents (*b*, *c*). For control experiments cAMP-kinase was heat-inactivated twice for 3 min at 95°C . The resulting precipitate was homogenized by strong vortexing; this was repeated directly before use.

unspecific channels by binding to them rather than by inducing their phosphorylation.

Patch-clamp experiments⁶ with taste receptor cells, isolated from taste buds of the frog tongue, have shown that cAMP (applied intracellularly in micromolar or extracellularly in millimolar concentrations) causes a decrease in outward potassium currents². Forskolin and methyl xanthines elicit the same response². In current-clamp the response becomes noticeable as a depolarization accompanied by a rise in membrane resistance (Fig. 1*a*). As depolarization elicits action potentials in these cells^{7,8}, we suggested that cAMP is a messenger in taste reception². Whole-cell recordings showed initial zero-current potentials (V_0) near -60 mV (Fig. 1) and spontaneous depolarizations towards -50 to -40 mV within 5–10 min. This response required the presence of 5 mM ATP in the pipette-filling solution. Addition of $0.5 \mu\text{M}$ cAMP to the pipette solution or superfusion of the cell with 5 mM cAMP caused further depolarization, and reduction of K^+ currents². Typically the reversible response to extracellular cAMP increased with time (Fig. 1*a*) but saturated at 25 mV of potential change. Complete depolarization was obtained by blocking K^+ -currents with 10 mM Ba.

The inhibitor (PKI) of cAMP-dependent protein kinase is a small protein^{9,10} with a relative molecular mass of 22,500 (M_r 22.5K) for the monomer. When included in the pipette solution ($1 \mu\text{M}$), it abolished the response to cAMP, applied from the bath, about 15 minutes after breaking the patch (Fig. 1*b*). The delay is explained by the time required for PKI to diffuse into



the cell. Subsequently, the membrane potential stabilized close to the -40 mV value usually observed in the absence of added cAMP, and the membrane resistance decreased. This response is compatible with PKI inhibiting the cAMP-dependent blockage of K^+ -conductance. The specificity of PKI suggests that the blockage is effected by the cAMP-kinase (protein kinase A).

The purified 40K catalytic subunit of cAMP-kinase¹¹ was included in the pipette solution at $7 \mu\text{M}$ together with 5 mM ATP. After the spontaneous depolarization (Fig. 1*c*), the cell was at first responsive to 5 mM cAMP added by superfusion. About 20 min after breaking the patch, the cell depolarized further and lost its responsiveness to cAMP. The potential stabilized at a value 20 mV less negative than observed before addition of cAMP. This result is also compatible with cAMP requiring cAMP-kinase for its depolarizing action.

Currents were recorded from excised inside-out membrane patches at 0 mV exposed to a normal K^+ gradient (Fig. 2). Spontaneously switching, Ba^{2+} -blockable outward currents were observed, corresponding to channel conductances of 74 pS (7 patches), 44 pS (34 patches) and 22 pS (5 patches). The current-voltage relationships of the two larger channels (Fig. 3) are linear. For the predominant 44 pS channel the reversal potential of -70 mV indicates a K^+/Na^+ permeability ratio of 19. The voltage-dependence of the switching activity of this channel was not found to be pronounced in the $\pm 70 \text{ mV}$ range.

When active, the 74 pS channel tended to obscure the signals from other channels. But this channel was only seen when EGTA

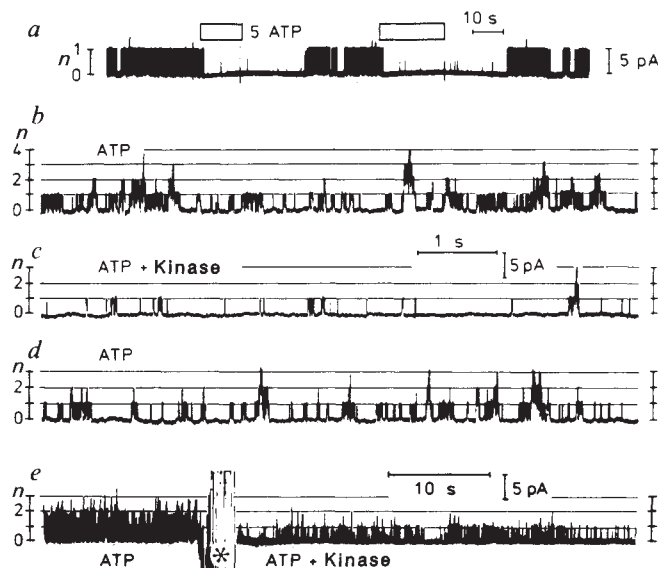


Fig. 2 Single-channel K^+ currents recorded from inside-out excised membrane patches at 0 mV. Opening events (current from bath to pipette, corresponding to membrane outward current) plotted upwards; n at ordinate, number of channels simultaneously open. Frequency limited to 2 kHz. High Na^+ solution in patch pipette: bath solution of Fig. 1. High K^+ bath solution (mM): KCl, 110; $MgCl_2$, 2; EGTA, 0.1 (0 in *a*); HEPES/KOH (pH 7.2), 10; Na_2ATP , 5 (0 mM ATP in part of *a*). *a*, Superfusion of the cytosolic surface of the patch with 5 mM ATP closed the dominant 74 pS K^+ channel (only one was active). This inactivation is due to a decrease of the free Ca^{2+} concentration. The channel was also blocked by the calmodulin inhibitor trifluoperazine (100 μ M). A 44 pS K^+ channel, also present but opening seldom, was not closed. The mean patch current (averaged over 20 s) decreased from 3.8 to 0.06 pA in response to the first addition of ATP shown. The single-channel open probability (P_o), calculated from the mean patch current, decreased from 0.7 to less than 0.01. Ba^{2+} (10 mM) blocked all K^+ channels from the cytosolic side (not shown); cAMP (5 mM) and Ca^{2+} (0.5 mM) were without effect (not shown). *b*, *c* and *d*, Records from the same patch. *b*, Control with 5 mM ATP before adding cAMP-kinase. In this patch at least four K^+ channels of 44 pS slope conductance were active. *c*, After stopping superfusion and pipetting 40 μ l of cAMP-kinase (dialysed stock, 26 μ M of catalytic subunit) into the bath (final concentration 6.6 μ M), the number of opening events and P_o were reduced. Observed for 7 min. *d*, Recovery; 7 min after washout of cAMP-kinase by superfusion P_o was increased again. *e*, Compressed timecourse of response to cAMP-kinase. Before addition of the enzyme, the mean patch current (averaged for 96 s) was 1.31 pA. At the time labelled with an asterisk 20 μ l of dialysed cAMP-kinase stock solution (15 μ M) was pipetted into the chamber (note electrical artefact) to a final concentration of 3 μ M. The mean patch current rapidly decreased and in the following 90 s it averaged 0.14 pA (0.39 pA in the first and 0.22 pA in the second period of 15 s each). During washout of the enzyme the patch current recovered slowly. After 4 min it reached 0.38 pA (averaged for 48 s), then the patch broke.

was not added to the bathing solution. When 5 mM ATP was added to the bath by superfusion, the 74 pS signal disappeared in all seven cases (Fig. 2*a*), whereas a 44 pS signal remained in three cases. The same reversible inactivation was obtained when 0.1 mM EGTA was used instead of 5 mM ATP. ATP did not close the 74 pS channel when added together with 0.5 mM $CaCl_2$. Thus the 74 pS K^+ channel is Ca^{2+} -activated. The regulatory range at pH 7.2 was estimated to 3 μ M of free Ca^{2+} (closed) to 30 μ M Ca^{2+} (switching). When using solutions of more than 3 μ M Ca^{2+} (no EGTA), it was convenient to close this channel using the Ca^{2+} -complexing action of ATP. Potassium channels of smaller conductance, whose phosphorylation by the cAMP-kinase required ATP, could then be observed more easily. The

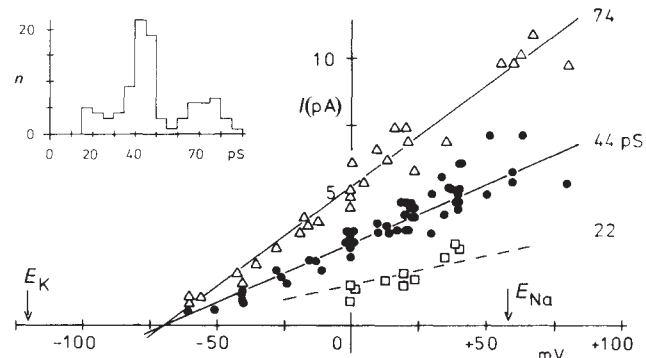


Fig. 3 Voltage dependence of single-channel K^+ currents recorded from 21 inside-out excised membrane patches. Solutions as in Fig. 2. Voltage sign as conventional for whole-cell data (cytosolic potential minus outside potential, here: bath minus pipette potential). For a reversal potential of -70 mV the calculated chord conductances (means $\pm$ s.d.) are 74 ± 9 pS (27 data points from 7 excised patches, all sensitive to Ca), 44 ± 6 pS (59 points, 11 patches of which 7 were sensitive to cAMP-kinase) and 22 ± 3 pS (10 points, 3 patches). E_K , Nernst-potential of K; E_{Na} , Nernst-potential of Na (does not apply to the 74 pS channel data, for which it was more positive). Inset, conductance histogram of the same data.

44 pS channel did not noticeably respond to changes in Ca^{2+} activity.

The three types of K^+ channels were active in the absence of cAMP. Addition of 5 mM cAMP or 0.5 mM $CaCl_2$ to the ATP solution did not change the switching activity of the predominant 44 pS K^+ channel. Thus neither Ca^{2+} nor cAMP is a gating ligand of this channel. This explains our previous findings that cAMP will not exert its depolarizing effect in whole-cell recordings in the absence of ATP², or when ATP is replaced by ATP- γ -S or AMP-PNP², or in the presence of PKI (Fig. 1*b*).

When purified catalytic subunit of cAMP-kinase was injected into a bathing solution containing 5 mM ATP, the opening rate of the 44 pS K^+ channel often decreased. Typically an initial decrease was noticeable within a few seconds (see Fig. 2*e*) and a further reduction occurred in the next minute. The high speed of response is in part explained by the vigorous stirring effect of injecting 20 or 40 μ l into a bath of 100 μ l. The high specific activity of the kinase (40 units ng^{-1}) and the high final concentration used (3–7 μ M) will also have contributed. In 15 of 24 patches where the approach was tried, the enzyme decreased the mean patch current due to 44 pS channels to a degree which made the fact obvious from a mere inspection of the records (Fig. 2*b, c*). Therefore, at least this K^+ channel can be inactivated by cAMP-kinase, explaining the depolarizing effect of cAMP² in whole-cell recordings. On washing the kinase away by superfusion, opening activity resumed within 1–2 min (Fig. 2*d*) in five of nine patches, presumably due to dephosphorylation effected by a membrane-attached protein phosphatase¹². In similar experiments (two of two trials) the seldom-observed 22 pS K^+ channel was also blocked by cAMP-kinase. Heat-inactivated kinase did not block K^+ channels in ten of ten control patches. In six of these patches, subsequent addition of active kinase reduced the patch current.

Thus at cytosolic calcium concentrations of less than 10 μ M a 44 pS K^+ channel predominates in excised patches. With normal ionic gradients across the patch this K^+ channel has a reversal potential near -70 mV, and may therefore contribute significantly to the cellular resting potential. In the presence of ATP, addition of the catalytic subunit of the cAMP-activated protein kinase to the cytosolic side of excised patches inactivates the 44 pS K^+ channel, presumably by phosphorylation. This reaction explains the membrane depolarization of ~ 25 mV and the increase in resistance caused by forskolin, methyl xanthines, cAMP and cAMP-kinase in whole-cell recordings. It also

explains the fact that the depolarization caused by cAMP requires ATP and is suppressed by PKI.

We expect that the observed depolarization facilitates the generation of action potentials^{7,8} and prolongs their duration. If the depolarization develops rapidly enough it may trigger action potentials, which could, in turn, trigger Ca^{2+} uptake (S. C. Kinnamon and S. D. Roper, personal communication), causing release of transmitter from sensory synapses at the basolateral membrane and repolarization by activating the Ca^{2+} dependent 74 pS K^{+} channel.

The recent finding of a sucrose-dependent GTP-dependent adenylate cyclase¹ implicates cAMP as cellular messenger for the 'sweet' sensation. Given taste cells of differing chemoreceptive specificity, cells sensing other qualities than sweet may also use cAMP for intracellular signalling. Elucidation of this point awaits the study of cAMP action in cells of known taste specificity. There are now indications that transduction of the qualities sour (S. C. Kinnamon and S. D. Roper, personal communication) and salty¹³ occurs by proton-modulated K^{+} channels and amiloride-blockable Na^{+} channels located at the apical membrane of taste receptor cells.

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Cyclic nucleotides may mediate taste transduction

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Taste stimulus adsorption is believed to occur at the taste cell microvillous membrane^{1,2}. But due to technical difficulties of inserting glass electrodes into the mammalian taste cell, little is known about the mechanisms of taste transduction^{3–12}. Reliable intracellular recordings are necessary to determine the characteristics of taste cells. This has been accomplished previously in the mouse^{8–12} and is reported here. Recent experiments indicated that cyclic nucleotides can act on the inner surface of the membranes of a variety of cells to alter their ion-channel activity^{13–22}, and these substances might act as intracellular transmitters in taste cells. But tight junctions found at the apical membrane of mammalian taste cells do not allow stimuli to enter the taste bud, making it difficult to alter the environment of the taste cell by perfusing with chemical solutions²³. Here we report that cyclic AMP, cyclic GMP, EGTA or tetraethyl-ammonium electrophoretically injected into the mouse taste cell induce membrane depolarization and increased membrane resistance. These results suggest that a cyclic nucleotide enzymatic cascade, modulated by calcium ions, may mediate the potassium permeability that controls taste, in a way analogous to visual and olfactory transduction.

To examine the possibility that internal transmitters play a role in taste transduction, we tried to alter the internal chemical

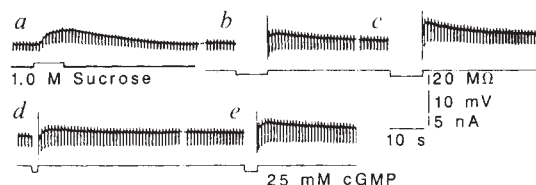


Fig. 1 Effects of electrophoretic injection of cGMP. Intracellular recording of the resting potential (-45 mV), response to sucrose stimulus (1.0 M), membrane resistance changes (*a*) and electrophoretically injected cGMP (25 mM) induced potentials from a cell (*b–e*). Cyclic GMP was injected with negative currents of 2.0 nA (*b*) and 3.0 nA (*c*) for 9 s, and with the negative currents of 3.0 nA for 2 s (*d*) and 4 s (*e*). The time shown by the broken lines was 30 s. Cyclic GMP was electrophoretically injected into a mouse taste cell from one barrel of a double-barrelled microelectrode, while the other barrel, filled with a 6% aqueous solution of Procion yellow, was monitoring membrane potential and membrane resistance. We could not record the membrane voltage during injections due to the large voltage drop across the electrode resistance.

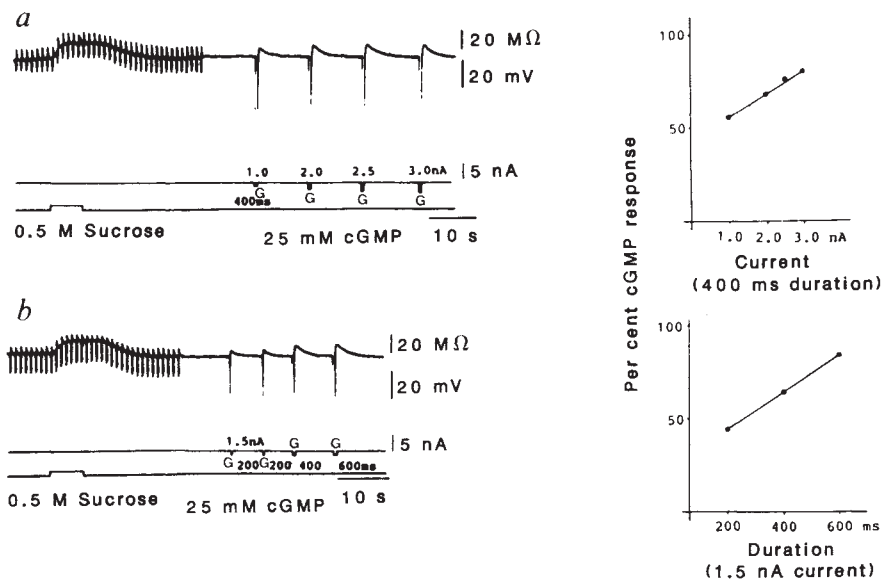
Methods. Adult mice (Slc:ICR) (30 – 50 g) were anaesthetized with pentobarbital sodium and tracheotomized. After the head was fixed in a holder, the tongue was gently extended with hooks onto a plastic plate. Tongues were adapted to constantly flowing distilled water (0.08 ml s^{-1}). Test solutions (0.08 ml s^{-1}) periodically replaced the distilled water rinse without interrupting fluid flow. Taste cells were stimulated with sucrose (0.5 M, 1.0 M). Conventional intracellular recordings were made with a high-impedance glass microelectrode (100 – 250 M Ω). An Ag–AgCl electrode placed into the musculature of the neck was used as an indifferent lead. The membrane resistance change of the taste cell was recorded with a bridge circuit and negative, 100 ms current pulses of 0.1 nA were passed from the recording electrode into the cell. Thus, a decrease in the magnitude of the electrotonic potentials produced by the test-current pulses correspond to a decrease in membrane resistance. The response was amplified with a d.c. amplifier and displayed on an oscilloscope. Responses were recorded on a frequency modulated (f.m.) magnetic tape and analysed at a later time.

constitution of the taste cell by intracellular electrophoretic injections. Cyclic AMP (25 mM), $5'$ -AMP (25 mM), cGMP (25 mM), $5'$ -GMP (25 mM), EGTA (100 mM), tetraethyl-ammonium (TEA) (500 mM), CaCl_2 (100 mM), KCl (100 mM) or NaCl (100 mM) was electrophoretically injected into mouse taste cells from one or two barrels of a double- or triple-barrelled microelectrode, while the other barrel, filled with a 6% aqueous solution of Procion yellow, monitored the membrane potential and membrane resistance. After recording the electrical responses, Procion yellow in the recording microelectrode was electrophoretically injected into the cell so that the location of the electrode tip could be determined. If a stained cell was not observed in the taste bud preparation, the responses were not included in the analysis.

Figure 1 shows the effects of cGMP on the taste cell. After a sucrose stimulus a membrane depolarization accompanied by an increase in membrane resistance was observed. Injection of cGMP produces the same effects. The amplitude of the depolarization is dependent on the applied electrophoretic current magnitude and duration. Figure 2*a* shows that progressively larger pulses of injected cGMP produce graded transient depolarizations of progressively larger amplitudes. Figure 2*b* shows that progressively longer pulse durations of injected cGMP cause the same effects. It has been suggested that cGMP mediates muscarinic acetylcholine action in various tissues^{13,14} and that increasing the intracellular concentration of cGMP produces a depolarization of the retinal rod membrane¹⁸. It has also been suggested that cGMP is the internal transmitter for phototransduction in cones and rods^{15–21}.

Figure 3 shows the effects of TEA injection. Sucrose taste

Fig. 2 Effects of electrophoretic injection of cGMP. *a*, Taste cell response to sucrose (0.5 M) and membrane voltage responses to injection of -1.0, -2.0, -2.5, and -3.0 nA of cGMP (25 mM) at 400 ms duration, left to right respectively. *b*, Another taste cell response to sucrose (0.5 M) and membrane voltage responses to injection of 200, 200, 400 and 600 ms duration of -1.5 nA of cGMP (25 mM), left to right respectively. Titration of cGMP response as a function of injection current. The plots (inset) show dose-response curves measured from Fig. 2*a* and *b* (Units: per cent cGMP response magnitude following stimulation with 0.5 M sucrose). G, cGMP.



stimulus produced a membrane depolarization accompanied by an increase in membrane resistance. TEA which is known as a potassium-channel blocker has the same effect when injected into the taste cell. The amplitude of the depolarization was dependent on the applied current magnitude and duration. The effects of a sucrose stimulus appear to be related to a decrease in conductance of the taste-cell potassium channels due to their closure. It is known that potassium channels of pancreatic cells close in response to glucose²⁴ and that the potassium channels of sympathetic ganglion cells of the frog close in response to a slow excitatory postsynaptic potential (e.p.s.p.)²⁵.

Intracellularly injected cAMP also induced depolarization accompanied by an increase in membrane resistance. The membrane depolarization caused by cAMP injection was always smaller than that caused by cGMP. Intracellularly injected 5'-AMP and 5'-GMP caused neither membrane polarization nor the membrane resistance change. Intracellularly injected EGTA induced a depolarization of the membrane and a membrane resistance increase. Intracellularly injected calcium or potassium ions induced membrane hyperpolarization and a decrease of the membrane resistance. The effects of injected EGTA and calcium ions into the taste cells suggest that calcium-mediated channels may be modulated by the taste stimulus and directly or indirectly cause taste transduction. However, the depolarization caused by EGTA injection was always smaller than that caused by cGMP. The effects of electrophoretic injection of sodium and chloride ions into the cell did not produce clear cut results. Effects of these compounds and drugs are summarized in Table 1.

These results suggest that a sucrose stimulus could have an indirect action on ion channels throughout the cell through the release of intracellular secondary messengers. Ionic channels in

other systems are known to be modulated by neurotransmitters acting through secondary messengers. For example, muscarine closes potassium channels in sympathetic neurones of the frog^{25,26} and serotonin closes potassium channels in sensory

Table 1 Effects of various chemicals administered by intracellular electrophoretic injection into mouse taste cells

Electrode filled with	Applied current polarity				No.
	Memb. pot.	Memb. res.	Memb. pot.	Memb. res.	
cGMP	0	0	Depo.	Increase	29
5'-GMP	0	0	0	0	5
cAMP	0	0	Depo.	Increase	12
5'-AMP	0	0	0	0	4
EGTA	0	0	Depo.	Increase	7
TEA	Depo.	Increase	0	0	5
CaCl ₂	Hyper.	Decrease	?	?	25
KCl	Hyper.	Decrease	?	?	12
NaCl	?	?	?	?	7
Total					106

Memb. pot., membrane potential; Memb. res., membrane resistance; Depo., depolarization; Hyper., Hyperpolarization; 0, no change; ?, doubtful.

neurones of *aplysia*²⁷, *helix*^{28,29} and the snail²⁹. The present experiments show that sucrose taste stimulus produces a membrane depolarization accompanied by a membrane resistance increase, and that cGMP or TEA produces the same effect. Our previous results suggested that sucrose response is due to a decrease of potassium conductance¹⁰. The data presented in this paper are consistent with the concept that when sucrose adsorbs to the receptor site of the taste cell, the internal cyclic nucleotide concentration is increased leading to a decrease in potassium conductance.

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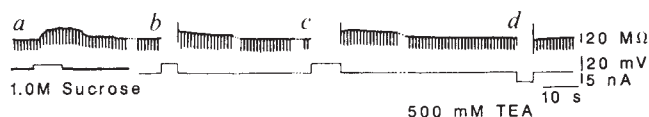


Fig. 3 Effects of electrophoretic injection of TEA. Intracellular recording of resting potential (-40 mV), response to sucrose stimulus (1.0 M), membrane resistance changes (*a*) and electrophoretically injected TEA (500 mM) (+5 nA) induced potentials from a cell (*b* and *c*). The time interval of broken lines was 30 s. TEA was injected with a positive current of 5 nA for 4 s (*b*) and 9 s (*c*). The last record was the injection into the taste cell with a negative current of 5 nA for 4 s. *d*, The symbols and methods are the same as described in Fig. 1.

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Tumour necrosis factor- α in murine autoimmune 'lupus' nephritis

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The (NZB $\times$ NZW) F_1 hybrid mouse develops a severe autoimmune disease similar to systemic lupus erythematosus in humans. Both the human and murine form of the disease show strong associations with alleles of the major histocompatibility complex (MHC) gene products^{1,2}. The severe form of the disease found in F_1 mice is due, in part, to dominant NZW gene(s) mapping with the H-2 complex (the murine MHC). Here we present evidence that the tumour necrosis factor (TNF- α) gene, which is located within the H-2 complex (the murine major histocompatibility complex), could be involved in the pathogenesis of lupus nephritis in F_1 mice. Thus, a restriction fragment length polymorphism in the TNF- α gene correlates with the reduced levels of TNF- α produced by NZW mice. Furthermore, replacement therapy with recombinant TNF- α induces a significant delay in the development of the nephritis.

NZW mice develop autoimmune haemolytic anaemia and mild glomerulonephritis³. NZW mice are phenotypically normal⁴. By contrast, most female (NZB $\times$ NZW) F_1 hybrids develop severe glomerulonephritis. By one year of age, 95% of animals die from nephritis. The NZW parent clearly makes a major genetic

contribution to the development of nephritis in F_1 mice. (NZB $\times$ NZW) F_1 $\times$ NZB back-cross studies have shown that the only dominant genetic contribution from NZW mice maps within the H-2 region, but may be separate from the class II loci⁵.

In an investigation of the *in vivo* effects of γ -interferon (IFN- γ) in the (NZB $\times$ NZW) F_1 lupus nephritis murine model, we have found that IFN- γ -treated animals have an accelerated development of glomerulonephritis⁶. As TNF synergizes with IFN- γ in a number of activities⁷, we have tested the *in vivo* effects of TNF- α in this murine model.

A group of 20 four-month-old female (NZB $\times$ NZW) F_1 mice was treated with 10 μ g recombinant murine TNF- α , three times a week intraperitoneally. Control mice received PBS solution. At four months of age F_1 mice show only minor disease. Treatment was given for a period of three months. Figure 1a shows the survival rate of mice treated with TNF- α . At 11 months 90% of treated animals were alive, whereas 80-85% of controls were dead. Even at 15 months (8 months after the treatment was stopped) 30% of treated mice were still alive, although all control mice were dead at 12 months. The development of proteinuria was also significantly delayed (Fig. 1b). No weight loss was observed in the treated group versus controls (not shown). A second *in vivo* experiment confirmed these results. Of the TNF- α -treated F_1 mice older than 11 months, 90% are alive, and 80% of the PBS-control mice are dead. The life-span of a BALB/c control group was not affected by a similar TNF- α treatment.

Recent cytogenetic studies have shown that the genes for human and mouse TNF- α and lymphotoxin (TNF- β) are located in tandem within the major histocompatibility complex⁸⁻¹¹. Therefore we have further investigated whether a TNF-encoding gene is involved in (NZB $\times$ NZW) F_1 lupus nephritis, by restriction fragment length polymorphism (RFLP) analysis.

A *Pvu*II 300-base-pair (bp) fragment derived from exon 4 of the murine TNF- α gene¹² was used as a probe to search for polymorphic restriction fragments. NZB mice, like BALB/c mice, show a *Bam*HI fragment of about 10 kilobases (kb) and the NZW mice show an 11.5-kb band. This polymorphism can also be demonstrated by *Bam*HI and *Hinc*II double digestion. Thus, NZW mice show a non-polymorphic 4.3-kb band and a polymorphic 2.7-kb band, in contrast to NZB mice with a 1.2-kb polymorphic band (Fig. 2a). No polymorphism could be demonstrated using a probe from murine TNF- β complementary DNA¹³ and 12 restriction enzymes (Fig. 2b). The autoimmune mouse strains MRL- +/+, MRL-1pr/1pr, B $\times$ SB and NOD all

Table 1 Murine TNF- α production by peritoneal exudate cells from various mouse strains

Cells per ml	Inducing agent(s)		Strain	(NZB $\times$ NZW) F_1		Balb/c
	LPS (μ g ml ⁻¹)	IFN- γ (U ml ⁻¹)		NZW	Cytolytic activity (U ml ⁻¹)	
5×10^5	—	—	NZW	10	8	9
4×10^5	0.1	—	NZW	44	11	51
4×10^5	1	—	NZW	53	14	50
4×10^5	0.01	10	NZW	49	11	43
5×10^5	0.01	10	NZW	78	14	61
5×10^5	—	100	NZW	64	15	72
5×10^5	0.1	10	NZW	92	15	104
5×10^5	1	100	NZW	126	17	121
5×10^6	1	100	NZW	230	19	192

Peritoneal exudate cells were obtained from mice primed 4-5 days earlier with 1 ml aged 3% thioglycollate broth (Difco) intraperitoneally. After a 2-h incubation at 37 °C, the nonadherent cells were removed by washing, and the adherent cells were collected, counted and transferred into empty wells at the given concentrations in RPMI 1640 medium, supplemented with 2 mM L-glutamine (Sigma), 100 IU ml⁻¹ penicillin, 100 μ g ml⁻¹ streptomycin and 5% heat-inactivated fetal calf serum. Adherent cells were activated for 16 h with recombinant murine IFN- γ (Genentech), lipopolysaccharide (LPS; Sigma) or simultaneously with both IFN- γ and LPS at the concentrations indicated, and the volume was adjusted to 1 ml in each well. After stimulation, the supernatants were collected, centrifuged and the cytolytic activities on L-M cells were quantitated *in vitro*¹⁵ (L-M cells) are a derivative of L929 murine connective tissue cells and were obtained from American Type Culture Collection CCL 1.2). In this bioassay the quantitation of cytolytic activity is based on the ability of TNF to lyse L-M cells in the presence of actinomycin D, as measured by crystal violet dye uptake of residual viable cells. Each data point represents the mean value determined twice from two separate mice per strain. Variation between mice from the same strain was always less than 10%.

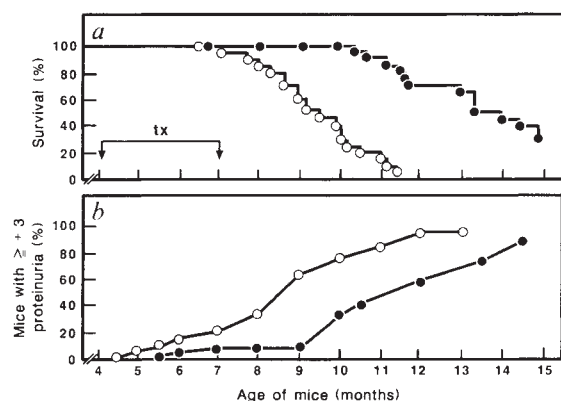


Fig. 1 *a*, Survival of (NZB x NZW)F₁ female mice treated with recombinant TNF-α. Murine TNF-α manufactured in *Escherichia coli* by recombinant DNA technology¹² (0.91 mg ml⁻¹; 2.8 × 10⁶ U per ml) was kindly provided by Dr H. M. Shepard. Mice received 10 μg per injection of TNF-α intraperitoneally, three times weekly for a period of 3 months, starting at the age of 4 months. The 20 female F₁ mice treated with TNF-α (●) are compared with age and sex-matched F₁ mice given PBS (○). Arrows indicate the time period of treatment. *b*, Cumulative frequency of significant proteinuria (≥3+) in (NZB x NZW)F₁ mice treated with TNF-α (●) compared to PBS-treated controls (○). To more accurately indicate the development of renal disease in all mice (alive and dead), a correction factor was induced. Thus, each point reflects the current level of proteinuria as well as the last measurement of proteinuria in deceased mice. Proteinuria was measured colorimetrically by the use of Uristix (Miles Laboratories). This produces an approximation of proteinuria as follows: trace, 10 mg dl⁻¹; 1+, 30 mg dl⁻¹; 2+, 100 mg dl⁻¹; 3+, 300 mg dl⁻¹; 4+, 1000 mg dl⁻¹. Measurements of proteinuria were performed by an observer who had no knowledge of the treatment given.

show the same TNF-α RFLP pattern as the NZW mice, but some non-autoimmune strains have this pattern as well¹⁴.

Next, we tested TNF-α production by peritoneal exudate cells obtained from NZB, NZW, (NZB x NZW)F₁ and BALB/c mice using an *in vitro* bioassay which quantifies the cytolytic activity of TNF-α (ref. 15). NZB and BALB/c mice show very similar cytolytic activities in all experiments (Table 1). NZW mice show very low TNF-α biological activity, regardless of the method used for activation. The F₁ mice show an intermediate activity, but TNF-α concentrations were much closer to the NZW parent than to the NZB parent (Table 1). At present we have no explanation for the fact that F₁ mice have TNF-α activity which is less than the mean level of that of the parental strains.

In contrast to the large differences in production of TNF-α, all strains tested produce similar quantities of IL-1. For example, 5 × 10⁵ peritoneal adherent cells activated with 100 U IFN-γ and 1 μg lipopolysaccharide for 16 hours induced the production of 109 U ml⁻¹, 114 U ml⁻¹, 98 U ml⁻¹ and 121 U ml⁻¹ IL-1 by NZB, NZW, F₁ and BALB/c mice, respectively.

Our data suggest that the NZW parent may contribute to the multigenic lupus-like autoimmune disease in (NZB x NZW)F₁ mice by donating a TNF-α gene capable of producing only limited amounts of TNF-α. The RFLP coincides with the degree of inducibility of TNF-α in the mice tested. Replacement therapy with recombinant TNF-α induces a significant delay in the development of the disease, suggesting that the treatment supplements the low TNF production from a possibly abnormal TNF-α gene contributed by the NZW parent.

The opposite *in vivo* effects of TNF-α and IFN-γ in (NZB x NZW)F₁ mice appears paradoxical. A broad range of TNF activities has been identified, some of them synergistic with IFN-γ (refs 7, 16). As most of these were *in vitro* studies, their

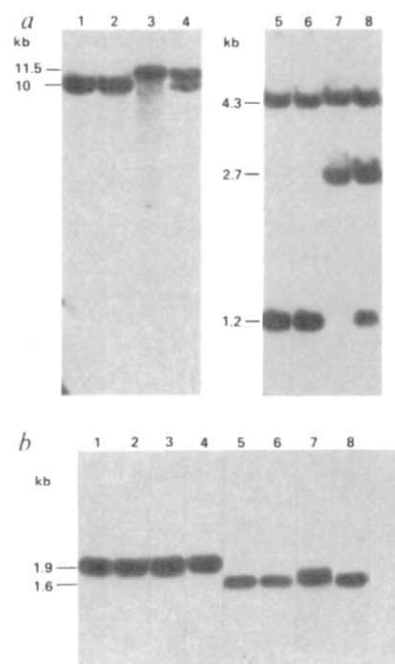


Fig. 2 Southern analysis of spleen DNA with *a*, murine TNF-α probe and *b*, murine TNF-β probe. Lanes 1 and 5 show DNA from BALB/c mice; lanes 2 and 6, from NZB mice; lanes 3 and 7, from NZW mice; and lanes 4 and 8 from (NZB x NZW)F₁ mice. DNA was digested with BamHI (lanes 1-4) and with BamHI-HincII (lanes 5-8). The probes used for this study include a PvuII 300-bp fragment of murine cDNA TNF-α (ref. 12) and the KpnI-XbaI 871-bp fragment of murine TNF-β cDNA (ref. 13) (gifts from Dr H. M. Shepard, Genentech). Size markers in kilobases (kb) are shown to the left.

Methods. Spleen DNA was isolated from the different mouse strains according to standard methods. DNA (10 μg per sample) was digested to completion with the different restriction enzymes according to the supplier's suggested conditions (Biolabs Inc.). DNA samples were run for 18-24 h on 0.7-0.9% agarose gel and Southern blotted for 20 h by conventional techniques. Filters were baked at 80 °C for 2.5 h and pre-hybridized at 42 °C in 50% formamide, 2% SDS, 3 × SSC (0.3 NaCl, 30 mM sodium citrate), 200 μg ml⁻¹ salmon sperm DNA and 10 × Denhardt's solution for 6-12 h. Hybridizations were in 50% formamide, 1% SDS, 3 × SSC, 200 μg ml⁻¹ salmon sperm DNA and 1 × Denhardt's solution. The probes were gel-purified and hexamer-primed to a specific activity 1.0 × 10⁸-1.0 × 10⁹ c.p.m. μg⁻¹ DNA. Filters were hybridized with 5 × 10⁵ c.p.m. ml⁻¹ for 18-24 h and washed in 2 × SSC, 0.1% SDS at room temperature, then at 60 °C in 0.1% SSC, 0.1% for 1 h, and then exposed for 3-7 days with XAR Kodak film.

biological relevance is unclear. The (NZB x NZW)F₁ system could be an important *in vivo* model for improving understanding of the physiological function of TNF. Furthermore, the results presented here have possible implications in the treatment of systemic lupus erythematosus in man.

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Cyclic AMP-dependent protein kinase opens chloride channels in normal but not cystic fibrosis airway epithelium

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Chloride (Cl^-) secretion by the airway epithelium regulates, in part, the quantity and composition of the respiratory tract fluid, thereby facilitating mucociliary clearance. The rate of Cl^- secretion is controlled by apical membrane Cl^- channels¹. Apical Cl^- channels are opened and Cl^- secretion is stimulated by a variety of hormones and neurotransmitters that increase intracellular levels of cyclic AMP (cAMP)¹⁻². In cystic fibrosis (CF), a common lethal genetic disease of Caucasians, airway^{3,4}, sweat-gland duct⁵, secretory-coil⁶ and possibly other epithelia⁷ are anion impermeable. This abnormality may explain several of the clinical manifestations of the disease. The Cl^- impermeability in CF-airway epithelia has been localized to the apical cell membrane⁴, where regulation of Cl^- channels is abnormal^{8,9}: hormonal secretagogues stimulate cAMP accumulation appropriately but Cl^- channels fail to open. Here we report that the purified catalytic subunit of cAMP-dependent protein kinase plus ATP opens Cl^- channels in excised, cell-free patches of membrane from normal cells, but fails to open Cl^- channels in CF cells. These results indicate that in normal cells, the cAMP-dependent protein kinase phosphorylates the Cl^- channel or an associated regulatory protein, causing the channel to open. The failure of CF Cl^- channels to open suggests a defect either in the channel or in such an associated regulatory protein.

cAMP-dependent protein kinase is composed of regulatory and catalytic subunits¹⁰. When cAMP binds to the regulatory subunit the catalytic subunit dissociates and phosphorylates target proteins. To determine if cAMP-dependent protein kinase regulates the Cl^- channel, we used excised, inside-out patches of membrane from normal human airway epithelial cells. The Cl^- channels present in these membrane patches have been identified as apical membrane Cl^- channels by their anion selectivity, by their response to agents that stimulate Cl^- secretion, by their inhibition by Cl^- channel blockers, and by their defective regulation in CF^{2,8,9}. The membrane potential was held at values between 0 and -40 mV and stepped to +40 mV

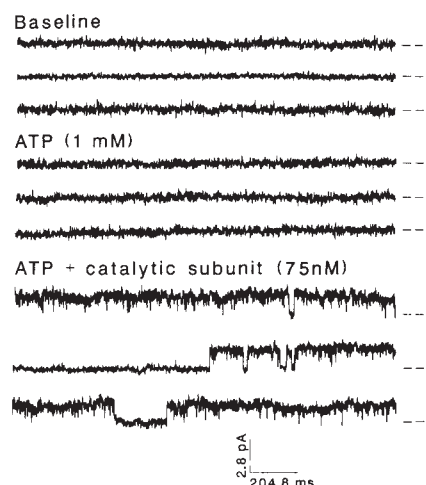


Fig. 1 Effect of the catalytic subunit of cAMP-dependent protein kinase on a Cl^- channel from a normal airway epithelia cell. Tracings are from an excised, inside-out patch. Outward current is shown as an upward deflection; the current level when channels were closed is shown by the dashed line. All tracings were obtained during 2 s steps to +40 mV; a pulse was given once every 6-8 s, the holding potential (V_H) was -40 mV. During the first 4 min after excision, 'Baseline', no channels opened. After adding 1 mM ATP to the internal solution, no channels opened during 5 min of observation. Subsequent addition of 75 nM purified catalytic subunit (specific activity 10-15 $\mu\text{M min}^{-1} \text{mg}^{-1}$) activated the channel. This concentration is less than the concentration of cAMP-dependent protein kinase observed in normal cells (200-700 nM)¹³.

Methods. The single-channel patch-clamp techniques used were similar to those previously described^{2,14}. A List EPC7 amplifier was used for current amplification and voltage clamping, and a laboratory computer system (Indec System) was used for data acquisition and analysis. Current amplitude and open-channel probability were determined from amplitude histograms. Currents were filtered at 1 kHz and the analog signal from the amplifier was digitized at a rate of one point every 500 μs . Pipet resistance was 2.5-6 M Ω and seal resistance was 2-25 G Ω . Pipet solution contained 140 mM NaCl, 2 mM CaCl_2 , 2 mM MgCl_2 , and 10 mM HEPES (pH 7.4 with NaOH). Bath (internal) solution was identical except that CaCl_2 was 0.062 mM with 1 mM EGTA (estimated free Ca^{2+} concentration 10 nM) and pH was 7.2. Similar results were obtained in a few studies in which Ca^{2+} concentration was 100 nM. Experiments were performed at room temperature (21-23 °C) on cells 1-3 days after plating. We studied isolated cells, cells at the edge of a cluster of cells, and cells in the middle of confluent areas of cells as previously reported^{2,8,9}. Voltages are reported in reference to the external surface of the membrane and outward (+) current refers to the flow of cations from internal to external surface of the patch, or anion flow in the opposite direction. Tracheas and nasal polyps from CF and non-CF subjects were obtained and the cells isolated and cultured as previously described⁸. The catalytic subunit of cAMP-dependent protein kinase was prepared from bovine heart as previously described¹⁵.

for 2 s every 6-8 s. If a Cl^- channel did not open during the first 4 min of observation, purified catalytic subunit of cAMP-dependent protein kinase and ATP were added to the internal, cytosolic solution. Figure 1 shows an example in which the combination of ATP and the catalytic subunit activated a Cl^- channel. Similar results were found in 10 cells from five normal subjects (Table 1).

In 8/10 successful cases, catalytic subunit activated the channel in less than 3 s following addition (complete addition of catalytic subunit required 20 s). In the other two cases the channel opened 10 and 40 s after completing addition of the kinase. Once a channel had been activated by the catalytic subunit, the probability of observing the channel in the open

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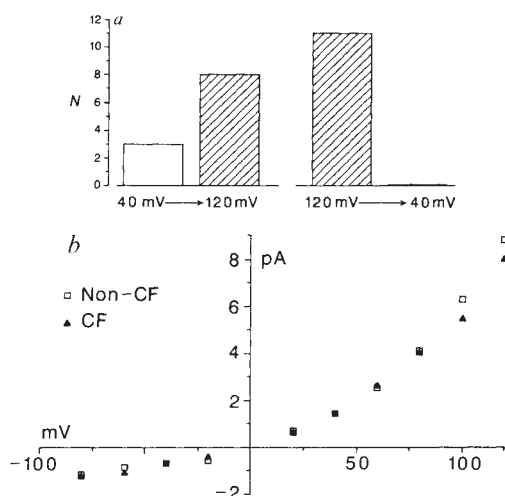


Fig. 2 Effect of membrane voltage on channel opening (*a*) and single-channel current in normal cells (*b*). *a*, Membrane voltage was held at 0 mV and two protocols were used. In one protocol the patch was depolarized to +40 mV for 25 s pulses, then to +120 mV for 25 s pulses. In the other protocol, +120 mV was the first voltage followed by 40 mV. The figure shows the number of channels that first opened at each voltage with the two protocols. Cl^- channels were identified by their characteristic size and current-voltage (*I-V*) relationship. The number of channels initially observed to open at +120 mV was significantly greater than the number initially opening at +40 mV ($P < 0.001$ by χ^2 analysis). *b*, Single-channel *I-V* relationship of a Cl^- channel from a normal cell (open symbols) (channel shown in Fig. 1) and a CF cell (closed symbols) (channel shown in Fig. 3).

state at +40 mV was 0.60 ± 0.05 ($n = 6$). Addition of either catalytic subunit alone ($n = 2$) or ATP alone ($n = 1$) (see Fig. 1) did not activate Cl^- channels, even though channels were subsequently shown to be present in the patch by addition of the complementary substance.

In 18 patches from normal cells, no Cl^- channel opened after ATP plus catalytic subunit (Table 1). To determine if a channel was present in the patch, we progressively depolarized the membrane. Despite large depolarizations, we never saw a Cl^- channel open if one had not been activated with catalytic subunit and ATP. We depolarized the membrane because in preliminary experiments we observed that Cl^- channels in excised membrane patches were more likely to open after large membrane depolarization. To further examine this phenomenon, patches were excised from non-stimulated cells and the voltage at which the channel first opened was recorded. No catalytic subunit was added. Alternating protocols were used: in one protocol an initial series of pulses to +40 mV was followed by a series of pulses to +120 mV; in the other protocol the initial series was at +120 mV and the subsequent series was at +40 mV. Figure 2*a* and Table 1 show that in three patches, Cl^- channels first opened at +40 mV, whereas in 19 patches, channels first opened at +120 mV. If a channel did not open in a patch depolarized to +120 mV, a Cl^- channel was never activated on subsequent steps to +40 mV. CF cells studied with this protocol showed the same pattern: one Cl^- channel first opened during the initial series of voltage steps to +40 mV, whereas six initially opened during steps to +120 mV (Table 1).

These results indicate that large depolarizations increase the likelihood that Cl^- channels will open for the first time (activate) in excised patches. Once a Cl^- channel was activated by depolarization, it remained activated, even at hyperpolarized voltages, as previously noted². The way in which depolarization modifies Cl^- channels is unknown but a voltage of +120 mV is about 160–180 mV away from the normal membrane voltage,

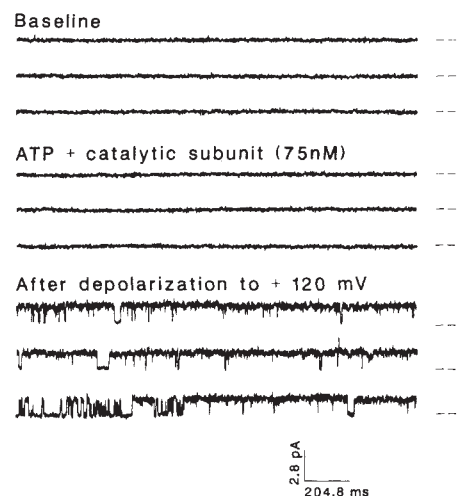


Fig. 3 Effect of catalytic subunit on a Cl^- channel from a CF cell. All tracings were obtained during 2 s steps to +40 mV from a holding potential of -40 mV. Pulses were given every 6–8 s. Conditions were the same as in the experiment shown in Fig. 1. The channel did not open during baseline conditions or following addition of 1 mM ATP and catalytic subunit 75 nM. Four minutes after addition of catalytic subunit we began to progressively depolarize the patch. The channel first opened at a voltage of +120 mV, 2 min after beginning the depolarizing steps.

creating a very strong electric field. This voltage gradient would set up a powerful force on polar and charged membrane molecules that could change the conformation of an ion channel or cause dissociation of some regulatory factor. This effect of voltage is probably not of physiological significance because the apical membrane is not exposed to depolarizing voltages¹. Nevertheless, the data suggest that large depolarizations can be used to detect Cl^- channels. Therefore we used depolarization as a measure of the presence of a Cl^- channel in a patch.

The current-voltage relationship was similar for channels opened with or without catalytic subunit; single-channel conductance was 31 ± 4 pS ($n = 9$, mean $\pm$ s.e.m.) for channels opened by catalytic subunit and 27 ± 1 pS ($n = 8$) for channels first opened by membrane depolarization. (Because the single-channel current-voltage relationship is not linear, there is no unique value of conductance. We report the slope conductance at 0 mV.) These results are consistent with our previous observa-

Table 1 Number of patches with Cl^- channels activated by ATP plus catalytic subunit and/or depolarization

<i>a</i> Catalytic subunit protocol				
Cells	Total patches	Patches with Cl^- channel activated by:		
		Catalytic subunit	Depolarization	Patches with no Cl^- channel
Normal	28	10	0	18
CF	41	0	18	23
<i>b</i> Depolarization protocol				
Cells	Total patches	Patches with Cl^- Channel activated at:		
		+40 mV	+120 mV	Patches with no Cl^- channel
Normal	51	3	19	29
CF	20	1	6	13

a, Patches were first exposed to catalytic subunit and then, if no channel opened, the membrane was depolarized. The protocol is the same as described in the legends to Figs 1 and 3 and in the text. *b*, The patches were depolarized as indicated in the legend to Fig. 2.

tion² that channels activated by secretagogue in the cell-attached mode had the same conductive properties as those that opened after excision from the cell. Catalytic subunit opened only a single type of Cl⁻ channel, which showed outward current rectification in symmetrical solutions (Fig. 2b). The results also suggest that an increase in cellular calcium (Ca²⁺) concentration is not required for channel opening. Catalytic subunit opened Cl⁻ channels when the calcium concentration of the internal bathing solution was 10 nM.

In CF airway cells, Cl⁻ channels are not activated by secretagogues such as isoprenaline, even though cellular levels of cAMP increase appropriately^{8,9}. This observation suggested that the abnormality in CF occurs somewhere between cAMP accumulation and Cl⁻ channel opening. To further localize the defect, we added catalytic subunit and ATP to the internal solution of excised, inside-out patches from CF cells. The conditions and voltage protocol were the same as for the normal cells shown above. Figure 3 shows an example of one cell in which catalytic subunit did not open a Cl⁻ channel. We waited 4 min after adding catalytic subunit and, when no channels opened, began to progressively depolarize the membrane. After depolarizing the patch to +120 mV, a Cl⁻ channel opened. Similar results were found in 18 cells from four CF patients (Table 1). The voltage required to induce the first channel opening was 109 ± 8 mV (mean ± s.e.m.). Once they were opened, CF Cl⁻ channels showed the same single-channel conductive properties as Cl⁻ channels from normal cells (Fig. 2b).

cAMP and cAMP-dependent protein kinase regulate a variety of ion channels in excitable cells (for reviews see refs 11 and 12). Our results using normal airway epithelial cells show that application of ATP plus the catalytic subunit of cAMP-dependent protein kinase mimics the effect of exogenous cAMP and secretagogues such as the β -adrenergic agonist, isoproterenol, in regulating Cl⁻ channels. Our data also indicate that regulation of CF airway Cl⁻ channels by cAMP-dependent protein kinase is abnormal. Defective regulation is distal to the hormone receptor and a defect in the post-receptor signal transduction pathway up through the cAMP-dependent protein kinase can not explain the abnormality. In conclusion, our results indicate that in normal airway epithelial cells the Cl⁻ channel protein or a regulatory protein closely associated with the channel is phosphorylated, resulting in channel opening, and that in CF epithelial cells this effector system is abnormal.

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Origin of the herbicide binding site of acetolactate synthase

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Acetolactate synthase (ALS) is the site of action of several new, structurally diverse classes of herbicides (sulphonylurea¹⁻⁴, imidazolinone^{5,6} and triazolo pyrimidine or sulphonanilide^{7,8}). These herbicides are unusual inhibitors in that they bear no obvious similarity to substrates (pyruvate and α -ketobutyrate), cofactors (thiamine pyrophosphate, FAD and magnesium), or allosteric effectors (valine, leucine and isoleucine) of this enzyme. They also interact in a complex way, in that time-dependent inhibition is observed^{1,6,8,9}, with tightest binding of the herbicide occurring under conditions of enzymatic turnover^{1,9}. Recently, the identity of the herbicide-specific site has been suggested by the discovery that the sequence of pyruvate oxidase¹⁰ is very similar to that of ALS¹¹⁻¹⁶. We propose that the herbicide-specific site of ALS is an evolutionary vestige of the quinone¹⁷ binding site of pyruvate oxidase. Consistent with this proposal, the ubiquinone homologues Q₀ and Q₁ are potent inhibitors of ALS, and Q₀, an imidazolinone herbicide (imazaquin), and a sulphonanilide herbicide, each compete with a radiolabelled sulphonylurea herbicide (sulphometuron methyl) for a common binding site on ALS.

ALS (EC 4.1.3.18), the first common enzyme of branched-chain amino-acid biosynthesis, has been identified¹⁻⁸ as the site of action of three new structurally distinct classes of herbicides. These three classes of herbicides were obtained by traditional screening methods and include sulphonylurea (exemplified by sulphometuron methyl and chlorsulphuron, the active factors in Du Pont's Oust and Glean), imidazolinone (exemplified by imazaquin, the active factor in American Cyanamid's Scepter) and triazolo pyrimidine or sulphonanilide herbicides (exemplified by Dow's 1,2,4-triazolo-(1,5-a)-2,4-dimethyl-3-(N-sulphonyl-(2-nitro-6-methylaniline))-1,5-pyrimidine, abbreviated here as 2-NO₂-6-Me-sulphonanilide) (see Fig. 1). It would appear that there is something unique about ALS that increases the likelihood of it being targeted by contemporary efforts to obtain commercial herbicides.

One of the more unusual features of ALS is its absolute requirement for FAD^{18,19}. The flavin is not required for the normal redox role typical of this cofactor, as reduction of the endogenous FAD, or its replacement by flavin analogues with higher or lower redox potentials (5-deaza-FAD or 8-chloro-FAD), has little or no effect on the rate of the enzymatic reaction, although the reduced enzyme is substantially less sensitive to inhibition by sulphometuron methyl (J.V.S. & C. Thorpe, unpublished observation). Sulphometuron methyl perturbs the visible absorption spectrum of the enzyme-bound FAD⁹, which, together with the effect of flavin oxidation state on herbicide sensitivity, suggests proximity of the isoalloxazine moiety of the cofactor and herbicide.

A possible explanation for ALS's unusual requirement for FAD, and its anomalous sensitivity to various herbicides, came with the discovery that the DNA sequence of *poxB*, the gene for pyruvate oxidase¹⁰, is very similar to that of *ilvB*, *ilvG* and *ilvI*, the genes for the large subunits of the three major isozymes of ALS in enteric bacteria (isozymes 1 (ALS1)^{11,12}, 2 (ALS2)¹³ and 3 (ALS3)¹⁴, respectively), as well as the genes for ALS from yeast¹⁵ and the higher plants¹⁶ *Arabidopsis thaliana* and

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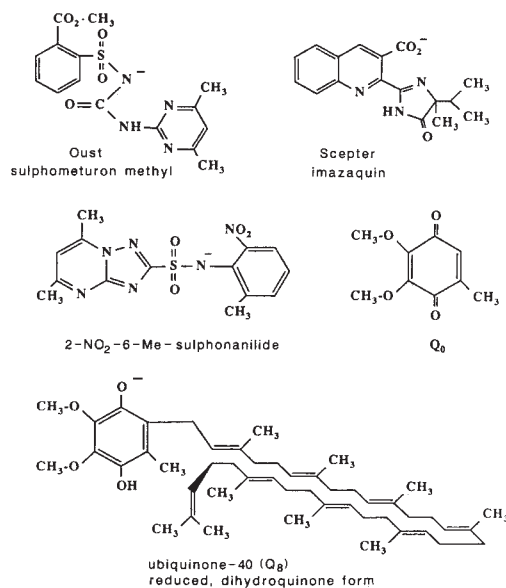


Fig. 1 The structures of sulphometuron methyl, imazaquin, 2-NO₂-6-Me-sulphonanilide, Q₀ and Q₈.

Nicotiana tabacum. Unlike ALS, pyruvate oxidase uses its required FAD for normal redox chemistry, and, *in vivo*, binds one additional cofactor, ubiquinone-40 (Q₈)¹⁷ (Fig. 1). There are some striking kinetic parallels between the mode of interaction of Q₈ with pyruvate oxidase and that of various herbicides with ALS. Pyruvate oxidase binds Q₈ (and a variety of lipids) most tightly in the presence of pyruvate^{17,20-25}, similar to the effect pyruvate has on ALS's affinity for sulphometuron methyl^{1,9}. Consideration of the expected chemistry of the pyruvate oxidase reaction would suggest that ubiquinone (in particular the quinone moiety) would bind to an equivalent region of the enzyme's active site with respect to the flavin, as does sulphometuron methyl in ALS⁹. Collectively, these observations would suggest that the herbicide binding domain of acetolactate synthase and the quinone binding site of pyruvate oxidase share a common evolutionary heritage.

The sensitivity of the three major bacterial isozymes of ALS to herbicides of each class (sulphonylurea, imidazolinone and sulphonanilide) is compared with their sensitivity to two water-soluble ubiquinone homologues (Q₀ and Q₁) in Table 1. All three of the bacterial isozymes are inhibited by Q₀ (Fig. 1) and Q₁ in a non-competitive way, although ALS1 and ALS2 exhibit greater sensitivity at low pyruvate (K_{is} and K_{ii} correspond to the inhibition constants for the slope and intercepts of double reciprocal plots, $1/v$ versus $1/S$ (where v is reaction velocity and S is the substrate, pyruvate, concentration) and represent the limiting inhibition constants at low and high concentrations of pyruvate, respectively). The isoprene moiety of Q₁ reduces the enzyme's affinity for it relative to Q₀, suggesting that the region of pyruvate oxidase that would accommodate the eight isoprene units of Q₈ (Fig. 1) is not shared by ALS.

Addition of Q₆ at concentrations above its solubility limit (100 and 20 μ M) to an ALS assay had negligible effect on the rate of the enzymic reaction. Inhibition of ALS1 and ALS3 by Q₀ is clearly time dependent, although only the initial phase of inhibition was characterized. Time-dependent inhibition was also observed for all of the herbicides examined with at least one of the isozymes of ALS.

Binding of [¹⁴C]-labelled sulphometuron methyl (1.3×10^4 c.p.m. nmol⁻¹) to ALS2, and competition by various unlabelled ligands, was monitored by equilibrium dialysis. These results are summarized in Table 2. The maximal amount of [¹⁴C] sulphometuron methyl bound by ALS was 0.35 mol per mol of

Table 1 Inhibition of acetolactate synthase by various herbicides (initial inhibition constants)

Isozyme: Inhibitor	ALS1 Inhibition constant (μ M)	ALS2 Inhibition constant (μ M)	ALS3 Inhibition constant (μ M)
Sulphometuron methyl*			
K_{is}	950	1.8	190
K_{ii}	4,300	9.0	310
Chlorsulphuron			
K_{is}	92	0.24	21
K_{ii}	350	6.9	240
Imazaquin†			
K_{is}	3,600	320	3,200
K_{ii}	1,300	600	1,600
2-NO ₂ -6-Me-sulphonanilide			
K_{is}	220	0.070	200
K_{ii}	160	0.78	160
Ubiquinone-0 (Q ₀)			
K_{is}	42	110	78
K_{ii}	100	150	370
Ubiquinone-5 (Q ₁)			
K_{is}	330	660	440
K_{ii}	740	400	1,100

Initial inhibition was determined under standard assay conditions¹⁸.

* The initial apparent inhibition constant (50 mM pyruvate) for ALS2 is 1.7 μ M; final, steady-state value is 0.082 μ M; and the maximal rate constant for transition is 0.15 min⁻¹. Slowly reversible inhibition is only observed in the presence of pyruvate^{1,9}.

† The initial K_i for ALS2 (50 mM pyruvate) is 800 μ M; the final K_i is 20 μ M; and rate of transition is 0.6 min⁻¹.

Table 2 Binding of radiolabelled sulphometuron methyl to ALS2 and the affinity of unlabelled ligands assessed by competition with radiolabel

Compound	K_D
Sulphometuron methyl*	14 μ M
Ubiquinone-0 (Q ₀)	520 μ M
Imazaquin	9,000 μ M
2-NO ₂ -6-Me-sulphonanilide	12 μ M

Equilibrium dialysis was conducted at 25 °C under conditions equivalent to those employed in enzymatic assays¹⁸, except for the omission of pyruvate.

* The concentrations of enzyme used to determine binding constants were 4.5, 11, 23, 45, 68 and 113 μ M protomer (69,000 g mol⁻¹). [¹⁴C]Sulphometuron methyl was varied at a fixed molar ratio of 2.8 to the concentration of enzymatic protomer employed.

protomeric unit (69,000 g mol⁻¹). Less than stoichiometric binding was also observed for [¹⁴C]thiamine pyrophosphate (L.M.C. and J.V.S., unpublished observation), with the amount of [¹⁴C]thiamine pyrophosphate bound by ALS2 declining in parallel with losses in enzymatic activity upon storage. For the ALS2 used in this study¹⁸ (specific activity = 19 μ mol acetolactate formed per min per mg), the maximal amount of [¹⁴C]thiamine pyrophosphate bound was 0.37 mol per mol, equivalent to the stoichiometry obtained for [¹⁴C]sulphometuron methyl. As the enzyme contains one equivalent of FAD per protomeric unit (two molecules of FAD per native enzyme dimer)¹⁸, the less than stoichiometric binding is unique to the herbicide and thiamine pyrophosphate. Presumably, the alterations in the enzyme leading to the loss of its ability to bind thiamine pyrophosphate have an equivalent effect on its ability to bind herbicide. The dissociation constant obtained for the [¹⁴C] sulphometuron methyl-ALS2 complex in the absence of pyruvate, 14 μ M, is substantially higher than the initial inhibition constant (1.8 μ M) and the final, steady-state inhibition constant (0.082 μ M), both of which are determined in the presence of pyruvate. This difference presumably reflects the higher affinity of the herbicide for ALS2 under turnover conditions (in the presence of pyruvate), which could not be maintained for

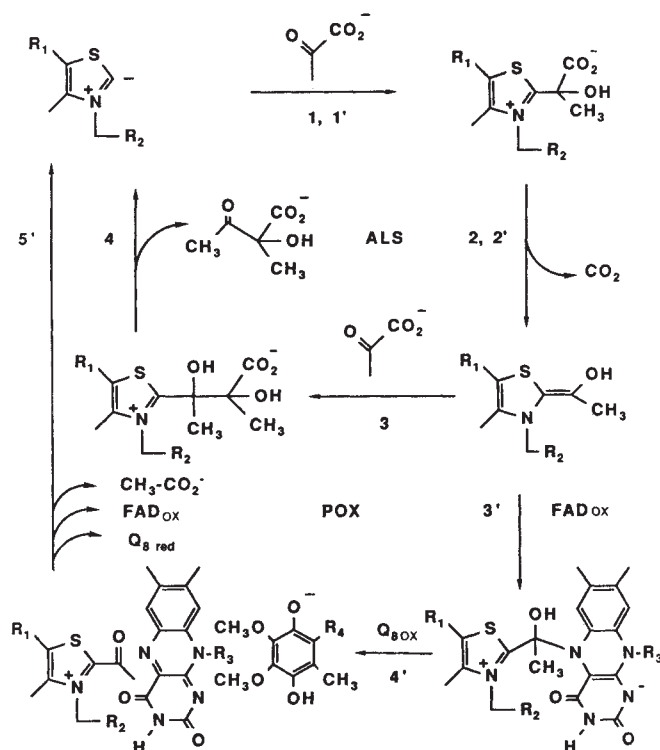


Fig. 2 Reaction schemes for ALS (steps 1–4) and pyruvate oxidase (POX, steps 1'–5'). The first two steps of either reaction (1 and 1', or 2 and 2') are likely to be the same. The groups R_1 , R_2 , R_3 , and R_4 , represent diphosphoethyl, pyrimidyl, adenosine-diphosphate ribityl moieties and the eight isoprene units of Q_8 , respectively.

the extended period of time required for equilibrium dialysis. Imazaquin, 2-NO₂-6-Me-sulphonanilide and Q_0 all displaced [¹⁴C]sulphometuron methyl from ALS2. At low concentrations of [¹⁴C]sulphometuron methyl (4 μ M), the unlabelled ligands could virtually abolish radiolabel bound to enzyme, and the apparent dissociation constant of [¹⁴C]sulphometuron methyl increased linearly with increasing concentrations of imazaquin, 2-NO₂-6-Me-sulphonanilide, or Q_0 , indicating that binding of these ligands to ALS2 is mutually exclusive.

Given the common evolutionary heritage of ALS and pyruvate oxidase, why might the former enzyme retain the requirement for FAD and the quinone binding site? Pyruvate oxidase effects the transfer of electrons between the ene-amine form of the hydroxyethyl-thiamine pyrophosphate intermediate (the product of step 2' in Fig. 2), flavin and quinone. To accomplish this effectively, it must shield the hydroxyethyl intermediate (functionally a carbanion) from solvent to prevent its protonation. As pyruvate oxidase is designed to protect the deprotonated hydroxyethyl intermediate, and the flavin is involved in its protection, there will be selective pressure to maintain the flavin in ALS. ALS2 cannot abstract the proton from carbon once protonation of the hydroxyethyl intermediate has occurred, nor can it abstract the proton from the hydroxyl to produce acetaldehyde (L.M.C. and J.V.S., unpublished observation). To change pyruvate oxidase into ALS, the electron transfer between the hydroxyethyl intermediate and flavin needs to be disfavoured, and a second keto-acid binding site interposed, to insure the proper reaction. If electron transfer between the hydroxyethyl intermediate and flavin does not occur, it is unlikely that ALS could function as a pyruvate oxidase (which it does not). Electron transfer between any flavin that may become reduced and quinone could be prevented by eliminating the lipid-binding domain of pyruvate oxidase, as, *in vivo*, quinone will be localized in membranes. ALS appears not to share the region of pyruvate oxidase that would accommodate lipids and/or the isoprene moieties of its natural cofactor, Q_8 , as Q_1 binds substantially less tightly than Q_0 to all three isozymes of ALS. As flavin might be expected to comprise at least part of the quinone binding site, and water soluble quinones (like Q_0 and Q_1) do not occur in abundance *in vivo*, there would be little selective pressure to eliminate the quinone site entirely as pyru-

vate oxidase (or their common ancestor) evolved into ALS. The possibility still remains, however, that the quinone/herbicide binding site of ALS has some as yet undiscovered regulatory or mechanistic role.

Identification of the herbicide binding site of ALS as an evolutionary vestige of a quinone site has some interesting ramifications in screening compounds for biological activity. There is a striking parallel between the diversity of structures identified that inhibit ALS and the equally diverse set of compounds (such as ureas and triazines) that inhibit photosystem 2 by virtue of binding to a plastoquinone site²⁶. It would seem that quinone sites are somewhat easier to select for by screening and offer a relatively low degree of structural stringency.

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Localized production of TGF- β mRNA in tumour promoter-stimulated mouse epidermis

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Tumour promoters induce a wide spectrum of morphological and biochemical alterations when applied to mouse epidermis *in vivo*¹. These include the induction of RNA (ref. 2), DNA (refs 3, 4) and protein synthesis^{5,6} during discrete phases of proliferation and differentiation⁷. This constitutes an ideal model for studying molecular events underlying the disruption of epidermal homeostasis by TPA, and its subsequent re-establishment. Transforming growth factor- β (TGF- β) can induce either growth stimulation, inhibition, or differentiation, depending on the target cell^{8,9}. A function has been proposed for TGF- β in wound healing¹⁰ and in tumour promotion¹¹, but the main source of TGF- β is generally thought to be platelets, macrophages or lymphocytes⁸, and a direct role for this growth factor in regulating tissue homeostasis *in vivo* has not been demonstrated. We show here that when the tumour promoter 12-tetradecanoyl-phorbol-13-acetate (TPA) is applied to the skin of mice, very high levels of TGF- β messenger RNA are induced in the epidermal cells. *In situ* hybridization techniques show that the main site of TGF- β synthesis is in the suprabasal differentiating epidermal cells. These results suggest that TGF- β may be a natural regulator of epidermal homeostasis which is important in tumour promotion.

Both TGF- β and the tumour promoter TPA can inhibit DNA synthesis in epithelial cells in a variety of systems^{8,12-15}. In addition, TGF- β stimulates differentiation of cultured human bronchial epithelial cells^{16,17}, whereas TPA has an apparently similar effect on murine or human keratinocytes *in vitro*^{13,14} and on mouse skin *in vivo*¹⁸. We therefore investigated the possible induction of TGF- β in mouse skin *in vivo* in response to TPA treatment. Total RNA was isolated from mouse epidermis at different times after treatment with TPA, and Northern blots were hybridized with a cloned TGF- β complementary DNA probe¹⁹. The results are shown in Fig. 1a. TPA induces a very strong initial stimulation of TGF- β mRNA production, which reaches a maximum about 6 hours after treatment. Further experiments have shown that an increase in TGF- β mRNA can be detected as early as 2–3 hours after treatment, indicating that this response is a relatively early effect (data not shown). Two mRNA species of ~2.5 and 1.9 kilobases (kb) appear to be coordinately expressed during the stimulation phase. Both mRNA species have been previously described¹⁹. The larger transcript is seen in a variety of cell types, and the smaller may represent an alternative form of processing. Rehybridizing the same Northern blot with a cDNA probe for the ubiquitous 7S RNA (ref. 20) (Fig. 1b) shows that the stimulation seen at 6 hours may even be underestimated in Fig. 1a, as this lane contains slightly less RNA than the control lane.

Comparison of Fig. 1a and b indicates that a second, but less intense, stimulation of TGF- β -related mRNA production may take place at ~48–72 hours after TPA treatment. Interestingly, there is a relative increase in the intensity of a larger transcript of ~4.3 kb which is only faintly visible at earlier time-points. Whether this represents a precursor form of the smaller species, or a transcript of a different related gene, remains to be established. Although the degree of stimulation seen at 48 hours was

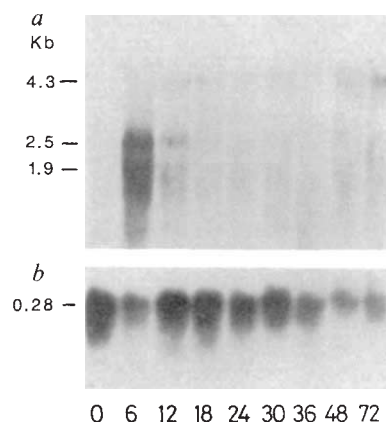


Fig. 1 Stimulation of TGF- β mRNA production in mouse epidermis by TPA. A Northern hybridization of total RNA from mouse epidermis at different times (in hours) after TPA treatment is shown, using *a*, TGF- β cDNA as probe¹⁹ and *b*, 7S RNA as probe for rehybridization²⁰. Size markers are shown on the left in kilobases.

Methods. The dorsal skins of 9–10-week-old female NIH mice were shaved and TPA (200 μ l 10⁻⁴ M solution in acetone) was applied topically. At specified times after TPA treatment the mice were killed by cervical dislocation. The dorsal skins were treated with a depilatory agent to remove hair after shaving, excised from the animal and incubated at 55 °C for 30 s before plunging into iced water. The epidermises were removed by scraping with a scalpel, quickly frozen in liquid nitrogen, and stored at -70 °C. Frozen epidermises were ground in liquid nitrogen and lysed in the presence of 5 M guanidinium thiocyanate. Total cellular RNA was isolated after centrifugation through CsCl as previously described²⁸. *a*, Aliquots (20 μ g) of total cellular RNA were electrophoresed through 1% agarose-formaldehyde gels, blotted onto nitrocellulose (Sartorius) and hybridized to the ³²P-labelled insert of the mouse TGF- β cDNA probe¹⁹. Blots were washed to a final stringency of 0.1 $\times$ SSC at 65 °C and exposed to Kodak X-OMAT film at -70 °C. Exposure time 3 d. *b*, The same blot re-hybridized with a probe for cytoplasmic 7S RNA. Exposure time 4 h.

not as high as the initial increase, it was further confirmed by the *in situ* hybridization results shown in Fig. 2.

The localization of the cells actively synthesizing TGF- β mRNA in skin was carried out by *in situ* hybridization. Comparison of control and TPA-treated skins shows, in agreement with the Northern blot in Fig. 1, that a very strong induction of TGF- β mRNA takes place within the epidermis (Fig. 2a–d and Fig. 3a, b). The level of expression in control epidermis is generally low (Fig. 2a), although in some sections a slight increase is seen in areas showing localized thickening. About 6 hours after TPA treatment, it is apparent from Fig. 2b and d that the induction of TGF- β mRNA occurs predominantly in the immediate suprabasal layer of the epidermis. This indicates that TGF- β production is initiated after basal cells have been committed to the terminal differentiation pathway. At 48 hours after TPA treatment, epidermal DNA synthesis is slowing down, and the substantially thicker epidermis shows signs of increased keratinization^{2-5,7}. TGF- β mRNA is raised with respect to controls at this time, and the transcripts again are localized in the highly differentiated cells of the upper epidermal layers (Fig. 2c).

An additional feature of the *in situ* hybridizations is that TGF- β mRNA production is stimulated in the hair follicles by TPA treatment (Fig. 3a and b). The level of TGF- β mRNA in the follicles of untreated skin was similar to that shown for interfollicular epidermis in Fig. 2a. The stimulation by TPA was not seen in the cells at the base of the follicle (Fig. 3c). Under the conditions of staining used, the hair bulb cells stain more densely than those in the upper areas of the follicles. Only the latter produce increased amounts of TGF- β mRNA, although

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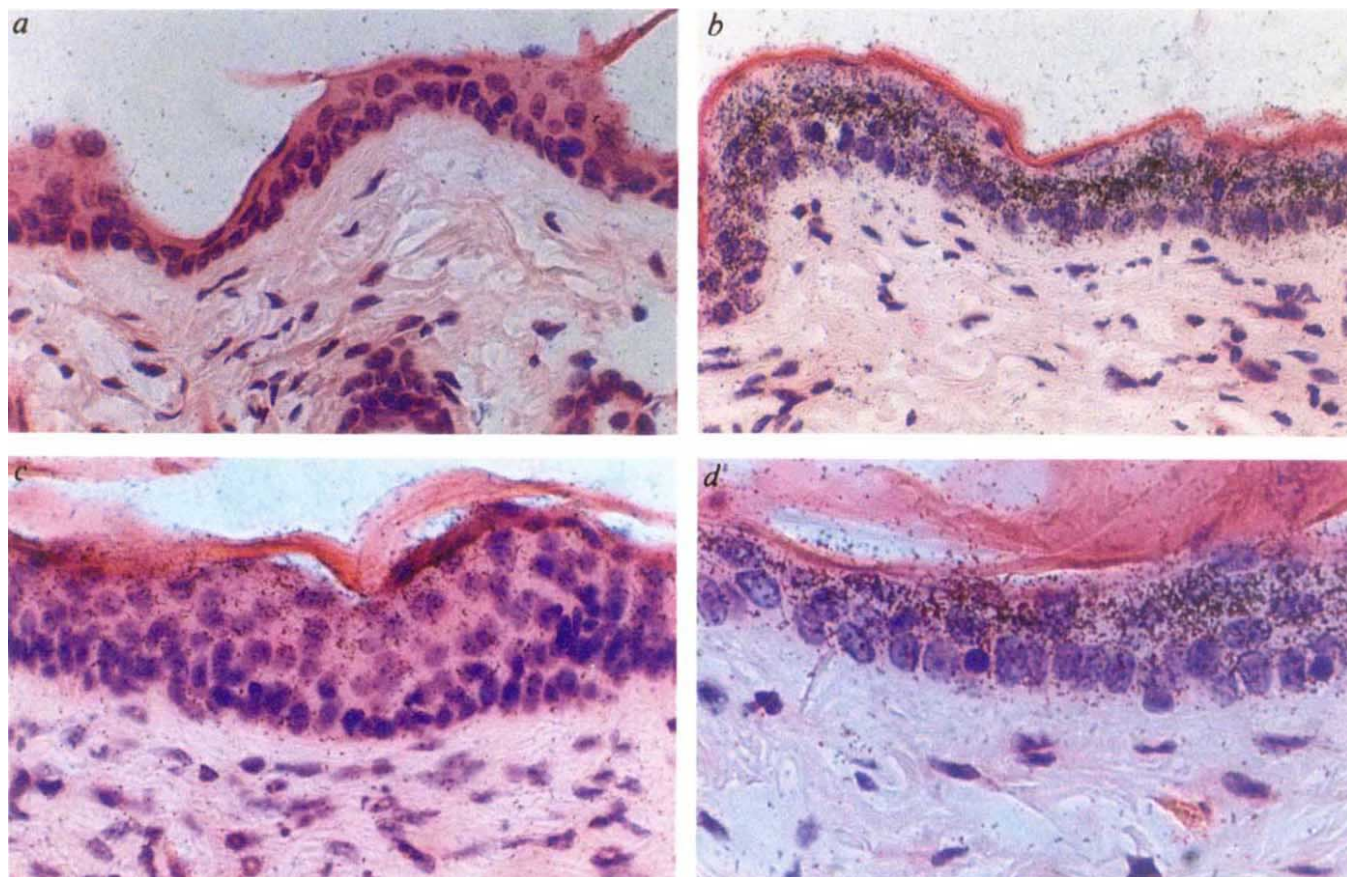


Fig. 2 Localization of TPA-stimulated TGF- β mRNA in skin sections by *in situ* hybridization. *a*, Normal mouse skin. *b* and *d*, 6 h after TPA treatment. *c*, 48 h after TPA treatment. Magnification: *a*, *b* and *c*, $\times 312$, *d*, $\times 500$. All sections were stained with H and E.

Methods. Mice were treated with TPA as described in the legend to Fig. 1. Skins were removed at the times shown, fixed in 4% paraformaldehyde and 5-micron-thick paraffin sections were hybridized²⁹ with a ³⁵S-labelled TGF- β riboprobe¹⁹. Exposure times shown were 3 weeks. A clear signal was obtained after a 2 d exposure of sections shown in *b* and *d*. Incubation of similar sections with a human chorionic gonadotropin probe as a negative control (provided by J. C. Fiddes) showed only background hybridization (data not shown).

a probe for the EGF receptor reacts equally well with both cell types (Fig. 3*d*). Whether this is related to a more basal cell character of the hair bulb cells, which may be a source of epidermal stem cells²¹, is not presently known.

The TGF- β polypeptide monomer has a relative molecular mass of 12,500 (12.5K) on reducing SDS-polyacrylamide gels⁸, is soluble in acetic acid⁸, and is relatively cysteine-rich¹⁹. Interestingly, a protein with similar characteristics was found to be highly induced in mouse epidermis by TPA treatment^{5,22}. At least part of this response may therefore be due to the production of TGF- β polypeptides, possibly as a component of a mixture of proteins of similar size, but further experiments involving both immunological and biological assays will be necessary to determine the levels of biologically active TGF- β produced.

An early response to tumour promoters which takes place 3–9 hours after treatment is a transient inhibition of basal cell DNA synthesis^{3,4} preceding the extensive stimulation observed during epidermal hyperplasia. As TGF- β has been shown to inhibit DNA synthesis in epithelial cells⁸, it is possible that this early response to TPA may be mediated by TGF- β , but confirmation of this must await the demonstration of biologically active TGF- β protein. Another early response to TPA is the induction of terminal differentiation¹⁸, but there is no evidence as yet that TGF- β can induce differentiation of keratinocytes¹⁵, in contrast to its effects on bronchial cells^{16,17} *in vitro*.

The late phase of TPA treatment is characterized by increased

keratinization and a reduction in DNA synthesis to control levels. TGF- β may also be involved at this stage since mRNA production is also increased in the upper epidermal layers. The existence of a diffusible negative regulator of growth which is produced by differentiating cells of stratified epithelia was postulated many years ago^{23–25}. TGF- β mRNA is produced in the appropriate cell type to act in this way, and may therefore be an endogenous regulator of epidermal homeostasis.

Many transformed epithelial cell lines do not respond to the negative effects of TGF- β (refs 15, 26). It has been proposed that initiation of mouse skin carcinogenesis results in a defective response to inducers of cell differentiation²⁷. Although it is not clear whether TGF- β can act as an endogenous inducer of cell differentiation *in vivo*, a function can be envisaged for TGF- β in tumour promotion, based on a differential response between normal and initiated cells to the growth inhibiting effects of this protein. Even a small decrease in the negative response to TGF- β after initiation could lead to selective inhibition of normal cells after each TPA treatment, eventually permitting the clonal expansion of initiated cells to form papillomas. But the process of tumour promotion is undoubtedly complex, and probably involves interplay of both negative and positive growth regulatory molecules still to be identified.

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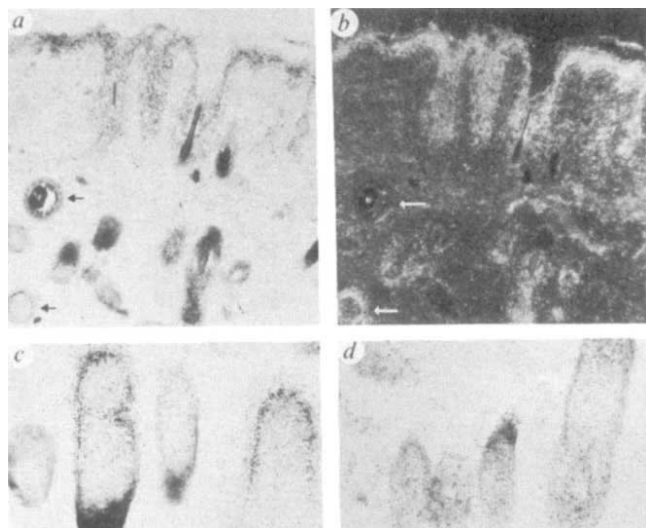


Fig. 3 Localization of TGF- β mRNA in follicular epidermis by *in situ* hybridization. Magnification in *a* and *b*, $\times 60$; *c* and *d*, $\times 150$. *a*, *b*, TGF- β mRNA shown extending into the hair follicles. The more densely stained follicle sections are from the base of the follicle, and are less highly labelled than sections from higher up (compare follicle sections marked with arrows). *c*, Higher magnification of follicles cut in section with the upper areas showing intense labelling. *d*, Similar section from the same skin showing more uniform hybridization to the EGF-receptor probe.

Methods. Sections were cut from mouse skin 6 h after TPA treatment. Procedures used were as described in the legend to Fig. 2, with the exception that the sections were lightly stained with Giemsa. *b* is a dark field image of the *in situ* hybridization shown in *a*. Probes used: *a*–*c*, TGF- β riboprobe; *d*, EGF receptor riboprobe (kindly provided by M. Hung).

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A highly basic histone H4 domain bound to the sharply bent region of nucleosomal DNA

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A nucleosomal core particle is composed of two each of histones H2A, H2B, H3 and H4 located inside the particle with ~ 147 base pairs (bp) of DNA wrapped around the octamer in about 1.8 turns of a left-handed superhelix^{1,2}. The path of the superhelix is not smooth; the DNA is sharply bent, or kinked, at positions symmetrically disposed at a distance of about one and four double-helical turns in both directions from the nucleosomal dyad axis (designated as sites ± 1 and ± 4 respectively³). This non-uniform bending is considered archetypal to other DNA-protein complexes, but its mechanism is not clear (reviewed in ref. 4). DNA-histone chemical cross-linking within the core particle has revealed strong binding of each of the two histone H4 molecules to DNA at a distance of 1.5 helical turns either side of the nucleosomal dyad axis (sites ± 1.5)^{5,6}. In each of these sites, a single flexible domain of H4 was previously shown to contact three points⁷, at about nucleotides 55 and 65 on one strand and nucleotide 88 on the complementary strand, numbering from the 5' terminus of each 147-base strand; these three locations are closely juxtaposed across the highly compressed¹ minor and major grooves (Fig. 1). Here we report that the amino-acid residue of histone H4 cross-linked at the 1.5 site is histidine-18, embedded in a highly basic cluster Lys-Arg-His-Arg-Lys-Val-Leu-Arg which is probably involved in the sharp bending of the DNA double helix at the ± 1 sites.

To locate the H4 peptide cross-linked at the 1.5 site the histones within the nuclei or core particles were covalently cross-linked to mildly depurinated DNA^{5,8}. The DNA moiety of the cross-linked complexes was then trimmed by acid and/or nuclease hydrolysis to the covalent tag of the form 5'-OH-d(Py)pR-protein, where Py is pyrimidine and R is the deoxyribose modified by cross-link to protein. ³²P label was introduced into the tag by polynucleotide kinase and [γ -³²P]ATP (ref. 17). Trypsin-generated nucleotide-peptide(s) of the cross-linked histone were separated by gel electrophoresis, and purified and identified.

In the map of histone H4-DNA contacts within the nuclei and isolated core particles (Fig. 2, lanes 1 and 2) the most abundant cross-link is represented by a doublet band of peptide T5, shown to be cross-linked at the 1.5 sites of nucleosomal DNA⁷. The band duplication is affected by the presence of cytosine and thymine in the nucleotide tags^{7–9}, and the faster (cytosine) band can be transformed into the slower band by cytosine conversion into uracil by bisulphite deamination (data not shown).

To improve homogeneity, we reconstituted the nucleosomes from the octameric histone complex and synthetic alternating polynucleotide poly[d(A-T)]·poly[d(A-T)] (refs 10, 11). Patterned of the cross-linked peptides of H4 (Fig. 2, lane 3) reveals a single prominent band corresponding to the upper thymine band of T5. Fainter bands can be seen on much longer exposures.

Upon cross-linking of isolated histone H4 to poly([d(A-T)]·poly[d(AT)]) (Fig. 2, lanes 4 and 6), deproteinized calf-thymus DNA (Fig. 2, lane 7), and to depurinated dinucleotides d(T-G) and d(C-G) (lanes 5 and 8, respectively), several new bands can be seen in the map in addition to (one of the) T5 bands.

Cross-linking of dinucleotides to proteins is a convenient tactic for isolation of model nucleotide-peptides in quantities sufficient for conventional peptide analysis. The only residue detected by amino-acid analysis of T5 is arginine. The relative



Fig. 1 The location of histone H4 cross-links within the core particle at the 1.5 site. A single H4 domain cross-links to nucleotides 55 and 65 (± 2) of one strand and nucleotides 88 ± 2 of the complementary strand, numbering from the respective 5' termini⁷. These three locations are juxtaposed at site 1.5 facing inwardly-located histones. The course of only one half of the nucleosomal superhelix is redrawn from ref. 1. The molecular dyad axis is horizontal, passing through the middle of core particle DNA at site 0 (nucleotides 73–74 of either strand).

molecular mass (M_r) of T5 as determined by mass spectrometry is 733 (Fig. 3). These data unambiguously identify the peptide as His-Arg, cross-linked through histidine-18 (Fig. 4).

By using combinations of specific H4 cleavages and amino-acid side chain modifications, it was shown that peptide T5 is located within residues 17–45 of the H4 molecule and is preceded by arginine and contains arginine as carboxy-terminal residue¹², in complete agreement with the above identification. Among other model peptides identified directly by amino-acid analysis and mass spectrometry, peptide T6f corresponds to cross-linking via histidine-75 (residues 68–78) and the peptide T11 to cross-linking via lysine-31 (residues 24–35) (details to be published elsewhere).

There are quite a few residues in H4 that are competent to react, as evidenced by maps of dinucleotide-H4 cross-links (Fig. 2, lanes 5 and 8), although individual reactivities differ due to the microenvironment. Also, our present protocol kinetically favours cross-linking through histidines rather than lysines (manuscript in preparation). Therefore, our results do not exclude binding of, for instance, Lys-16 or Lys-20 at the 1.5 sites or of other lysines elsewhere in the nucleosome. But comparison of the maps in Fig. 2 with His-18 as a standard, shows His-75 (peptide T6f) of H4 free in solution to be as efficiently cross-linked to dinucleotides, less efficiently to DNA in isolated H4-polynucleotide complexes, and withdrawn from contact with DNA in core particle (compare with ref. 13).

Thus, at the nucleotides 55, 65 and 88 of 1.5 sites of sharp bending of nucleosomal DNA, the DNA interacts with the unique H4 peptide region encompassing histidine-18 within the exceptionally dense contiguous cluster of positively charged residues (Fig. 4). The cross-linked region marks the boundary between buried and exposed parts of H4 (ref. 14) and is a hotspot for postranscriptional amino-acid modifications (Fig. 4). The ensuing variability of this basic region in a close contact with DNA at a strategic point in the nucleosome suggests its involvement in a nucleosome-structure modulation (reviewed in ref. 15).

The DNA region in contact with H4 domain partly delineated above is singled out by an altered sensitivity to nuclease attack¹⁶; heightened reactivity to dimethylsulphate methylation at nucleotide 62¹⁷; some unstacking of base pairs detected biochemically at around nucleotides 88 and, to a lesser extent, 67 and 57¹⁸, confirming the crystallographic definition of sharp bends at sites ± 1 and ± 1.5 ^{1,2}.

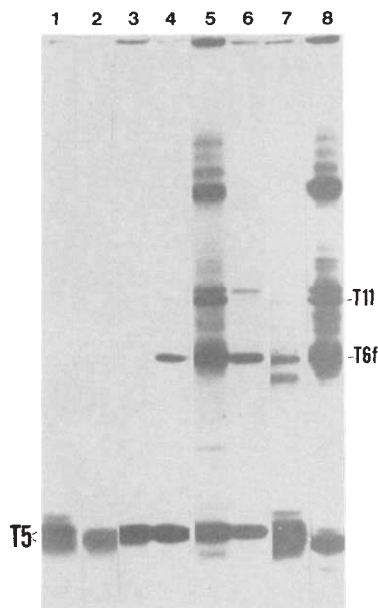


Fig. 2 Gel-electrophoretic mapping of ³²P-labelled cross-linked tryptic peptides of histone H4 at pH 8.6. H4 was cross-linked to DNA within the chicken-reticulocyte nuclei (lane 1), chicken-reticulocyte core particles (lane 2) and core particles reconstituted on poly[d(A-T)]·poly[d(A-T)] (lane 3). Other maps are of isolated H4 cross-links with poly[d(A-T)]·poly[d(A-T)] (lane 4), calf thymus DNA (lane 5), dinucleotide d(T-G) (lane 6) and dinucleotide d(C-G) (lane 8). All the above samples were labelled by [γ -³²P]ATP with polynucleotide kinase. In lane 6, the label was transferred to an isolated H4 by cross-linking H4 to poly[d(A-T)]·poly[d(A-T)] synthesized from dATP and [α -³²P]TTP, and the cross-linking was followed by acid hydrolysis of the polynucleotide. This shows that the band positions and relative intensities are not dependent on very different means of the DNA hydrolysis and introduction of the label (compare with lane 4).

Methods. Chicken reticulocyte nuclei were isolated as in ref. 26. Nucleosomal core particles were obtained as in ref. 27. Poly[d(AT)]·poly[d(AT)] was synthesized with DNA-polymerase Klenow fragment⁹. Nucleosome reconstitution starting with 45 A₂₆₀ U of poly[d(AT)]·poly[d(AT)] and 0.8 A₂₇₅ U of chicken-reticulocyte histone octameric complex was performed by step-gradient dialysis with subsequent micrococcal-nuclease digestion^{10,11}. Nucleoprotein complexes were cross-linked by methylation, depurination and borohydride reduction⁸, and were subjected to either exhaustive micrococcal-nuclease digestion only or formic-acid-diphenylamine hydrolysis, followed by bacterial alkaline phosphatase and micrococcal-nuclease treatments^{7,9}. The label was introduced into the nucleotide tag by the T4 polynucleotide kinase with [γ -³²P]ATP. Individually labelled histone H4 was isolated by preparative gel electrophoresis and treated with (tosyl-L-phenylalanine chloromethyl ketone)-trypsin additionally purified by reverse phase high performance liquid chromatography (RP HPLC). Labelled nucleotide-peptides were patterned in ultrathin 20% acrylamide, 0.67% methylene-bis-acrylamide-gel slabs by electrophoresis towards the anode in 12 mM Tris, 75 mM glycine, 0.1 mM EDTA, 0.2 mM thioglycolic acid, pH 8.6, at 60 V cm⁻¹ for 90 min and exposed to an X-ray film. Cross-linking of d(T-G) or d(C-G) to isolated H4 was as described in the legend to Fig. 3, except that the reactants were taken in equimolar amounts.

Striking examples of DNA bending by phage 434 repressor¹⁹ and *Eco*R1 endonuclease²⁰ show nucleotide-sequence-dependent²¹ DNA bends embedded in a complicated network of hydrogen bonding and electrostatic interactions. No stable hydrogen bonding was detected in the core particle^{17,22}. Sequence-dependent bending preferences of DNA influence the positioning of nucleosomes²³, but the actual distribution of putative kinks seems to be imposed by the histone-octamer

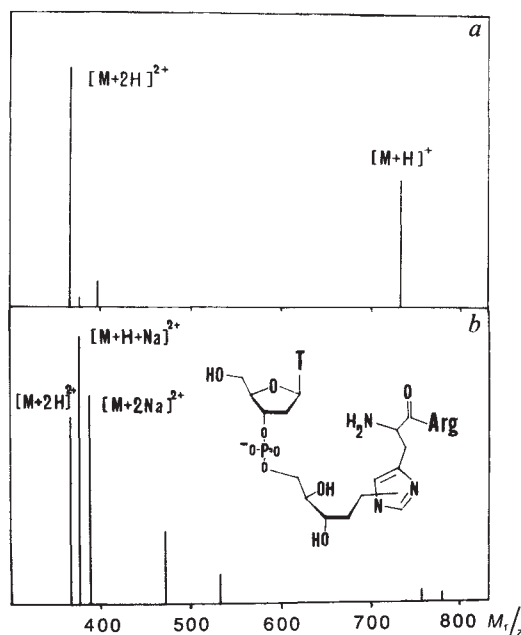


Fig. 3 ERIAD²⁸ mass-spectra of quasi-molecular ions of the 5'-dephosphorylated nucleotide-peptide T5 injected in the absence (a) or presence (b) of sodium ions. M_r , relative molecular mass; z , charge; H , proton; and M , compound under study. In a, the line of the quasi-molecular ion $[M+H]^+$ corresponds to a relative mass of 734 ($z=1$). The other line shows $M_r/z=367.5$ and should correspond to the doubly protonated form $[M+2H]^{2+}$ of M_r 735 and $z=2$. To confirm this, the injection in the presence of sodium ions was performed producing a triplet of lines with a mass increment of 11 (b), thus proving that lines with M_r/z of 735/2, 757/2 and 779/2 do indeed originate from the doubly protonated ($z=2$) forms $[M+2H]^{2+}$, $[M+H+Na]^{2+}$ and $[M+2Na]^{2+}$. Hence, the M_r of M is 733. There is one, and only one, nucleotide-peptide in the set of all possible trypsin cleavage products of cross-linked H4 that exactly conforms to this mass, and it is TpR-(His18-Arg19) (b). The precise structure of the imidazole-deoxyribose cross-link is presently under investigation.

Methods. Histone H4 was purified from an HCl extract of chicken reticulocyte chromatin by HPLC on a μ -Bondapak C₁₈ column with a 20–70% acetonitrile gradient in 0.1% TFA (trifluoroacetic acid); 500 nmol of the synthetic dinucleotide d(pTpG) were depurinated by heating in 70% formic acid at 70 °C for 20 min, freeze-dried twice, incubated with 100 nmol of histone H4 at pH 7.5 in a pH-stat for 4 h. The cross-links were reduced with 20 mM sodium borohydride in an ice-water bath for 2 h. Trypsin digestion was performed as described in the legend to Fig. 2. The trypsin digest was fractionated by RP HPLC on microbore μ -Bondapak C₁₈ column with 0–40% acetonitrile gradient in 0.1% TFA with triple-wave-length monitoring (at 210, 260 and 280 nm) to discern peptides from nucleotide-peptides. Crude nucleotide-peptide fractions were rechromatographed at pH 7.5 (50 mM Tris-TFA), fractions therefrom were dephosphorylated by bacterial alkaline phosphatase, rechromatographed under the same conditions, then rechromatographed in 0.1% TFA. Amino-acid analyses were performed on a Picotag amino-acid analyser (Waters). Mass spectrometry was performed on a mass spectrometer MS 3303 (NTO AN, USSR) by the method of extraction of ions from solutions (ERIAD)²⁸ with direct injection of samples in 30% methanol with or without 10 mM sodium borate. Small aliquots of the purified nucleotide-peptides were rephosphorylated with [γ -³²P]ATP and polynucleotide kinase and run in the pH 8.6 gels as described in a legend to Fig. 2, to confirm their identity.

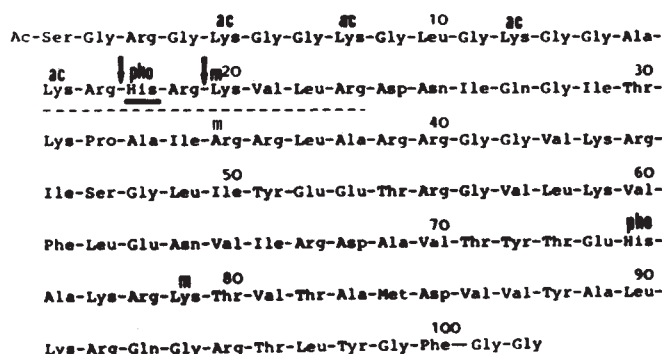


Fig. 4 Amino-acid sequence of chicken histone H4²⁹. Histidine-18 is underlined by a heavy line, the basic cluster by a broken line. Sites of post-transcriptional modifications are indicated by: ac, acetylation; m, methylation; pho, phosphorylation (ref. 15 and references therein). Note that out of 102 residues, a stretch of five contiguous residues in the neighbourhood of the cross-link accommodates three out of nine known modifications of H4. Vertical arrows show the boundaries of the trypsin-sensitive amino-terminal tail of H4 in the nucleosome¹⁴.

bending. The close proximity of the sharply bent DNA site to a highly basic amino-acid cluster supports the notion that such bending is affected electrostatically, perhaps by lateral neutralization of DNA phosphates^{24,25}. It remains to be determined what weight the reported interaction of the highly basic region of H4 has in the sum of other histone-DNA contacts that ensure folding of a nucleosomal DNA.

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structure, as evidenced by the nuclease protection pattern of core particle reconstituted on uniform poly[d(A-T)]·poly[d(A-T)] (ref. 11). The fact that unstacking¹⁸ is spread over roughly the same DNA locations as these cross-linked to the H4 domain, suggests the domain's probable involvement in sharp DNA

P-M hybrid dysgenesis does not mobilize other transposable element families in *D. melanogaster*

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Mobilization of the P family of transposable elements in *Drosophila melanogaster* occurs in the hybrid progeny of males from an element-bearing strain (P strain) and females from an element-free strain (M strain)¹. We tested whether the same crosses could mobilize other families of transposable elements. A mating scheme was used in which a set of X chromosomes was kept for 20 generations in either the active condition (known as hybrid dysgenesis) or the inactive condition (nondysgenic). Examination of 19 families of transposable elements by *in situ* hybridization indicated that only the P family was measurably mobilized under dysgenic conditions. Thus, P-M hybrid dysgenesis does not increase the transpositional activity of other families of transposable elements in *D. melanogaster*. We discuss possible explanations for several published reports to the contrary²⁻⁵.

The experimental design is shown in Fig. 1. All 18 lines initially had identical X chromosomes as they were descended from a single male. Any changes between G₀ and G₂₀ were necessarily the result of excision or insertion events. These events were detected by *in situ* hybridization of biotin labelled transposable element (TE) DNAs to salivary gland chromosomes as described in Fig. 1. P-element transposase activity was measured in each line every generation by determining the mutability of *sn^w*, a transposase-sensitive allele⁶⁻⁹. Figure 2 shows that high levels of *sn^w* mutability were observed in all 20 generations, confirming that throughout the experiment, high transposase activity was present in dysgenic but not in nondysgenic lines.

We measured excision rates in units of excisions per element-generation. Both complete and partial excisions are included provided the latter leave behind less than the minimum size detectable by our methods (~500 base pairs (bp)). Excision rates represent the average for all euchromatic elements on the X chromosome. (No excision estimates were possible for those TE families lacking X chromosomal sites.) The results (Table 1) indicate that P elements had a high excision rate in the dysgenic but not the nondysgenic lines. No other element in either set of lines had a significant frequency of excision. In contrast to most other elements, a large proportion of P element excisions are known to be partial losses which would not be detectable by our methods¹⁰⁻¹². Therefore, our conclusion that only P elements are induced to excise by P-M hybrid dysgenesis applies *a fortiori*.

Insertion rates were estimated as the number of insertions per donor element-generation. Insertion rates represent the average for only the euchromatic donor sites on all chromosomes. The results (Table 2) again show that only the P family was highly mobilized in the dysgenic lines, with an insertion rate at least 80-fold greater than any other TE family. Even the nondysgenic P elements were at least twice as mobile as any other TE family in either set of lines. We did observe a slightly greater absolute number of non-P-element events in the dysgenic group, especially for the element 297. But this difference may merely reflect the greater sample size in the dysgenic lines and Poisson variability. Unfortunately, uncertainties in the number of transposition opportunities precludes full statistical analysis of these data.

There are several reasons to think the difference between the P-element family and other TEs is even greater than suggested

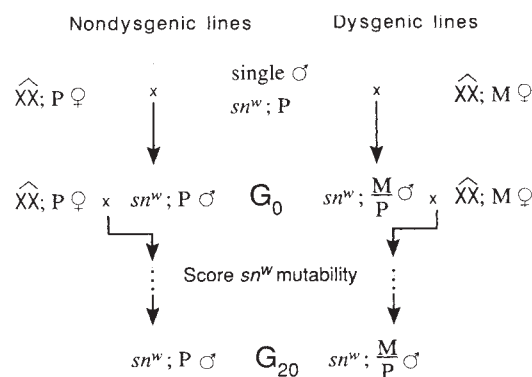


Fig. 1 The symbol M refers to the background genotype of an M strain bearing the recessive eye colour markers *bw* on the second chromosome and *st* on the third²⁸. P indicates the genetic background of π_2 , a P strain⁶. All females in this scheme carried a compound X (symbolized XX) chromosome. This results in the *sn^w* X chromosome being passed from father to son. A single *sn^w*; π_2 (P) male was doubly mated to XX females (C(1)DX, *yf*; π_2)^{6,28} of P cytotype to initiate nondysgenic lines and XX females (C(1)DX, *yf*; *bw*; *st*)²⁸ of M cytotype to initiate dysgenic lines¹. Eight nondysgenic and twelve dysgenic lines were maintained by repeated backcrossing of single *sn^w* males to the attached-X P or M females. The recessive markers, *bw* and *st*, were used to ensure retention of the π_2 autosomes in dysgenic lines. Every generation, all males in each line were scored for bristle phenotype, and *sn^w* mutation rates estimated as described⁶⁻⁹. Crosses were maintained at 21 °C. Only seven nondysgenic and eleven dysgenic lines survived to G₂₀ and are included in the results. At generations 0 and 20, salivary glands were prepared from male third instar larvae for *in situ* hybridization with biotin-dUTP-labelled cloned TE DNA (see Table 1) as described²⁹. The number and positions of elements for each TE family were determined in a single larva at G₀ and a single larva from each line at G₂₀ (see Tables 1 and 2).

by Table 2. First, most of the TEs we examined had label in the chromocentre, indicating the presence of heterochromatic copies, but P elements did not. This leads to an underestimate of the number of donor sites, and therefore an overestimate of the insertion rates of these elements relative to the P family. Also the number of P element donor sites is probably overestimated in dysgenic lines as a result of male recombination events on the autosomes during the course of the experiment¹³. As the dysgenic lines are repeatedly backcrossed to an M strain (Fig. 1), such recombination results in a net loss of P-element donor sites. Other families will increase or decrease with equal likelihood, depending on how many copies of the element are in the M strain. Thus, the insertion rate of P elements is probably underestimated relative to the average of the other families.

There have been several previous attempts to detect mobilization of other transposable elements by P-M hybrid dysgenesis^{2-5,14}. Some reports claimed that high levels of transposition and excision activity of non-P elements, including transposition 'bursts', occurred in response to P-M hybrid dysgenesis²⁻⁵. Unfortunately, these data did not distinguish between new mobilization events induced by hybrid dysgenesis versus pre-existing variability in the stocks. The lack of nondysgenic controls in some of these experiments leaves open the possibility that the transposition bursts are real, but are unrelated to the P-M system. It should be noted that these studies employed a variety of different strains. A more detailed discussion of alternate interpretations of these data is presented elsewhere¹. Finally, our data suggest that two other cases in which *copia* was mobilized^{15,16} are probably also independent of P-M hybrid dysgenesis.

Table 1 Excision rates in dysgenic and nondysgenic lines

TE family*	X sites†	Dysgenic lines			Nondysgenic lines		
		Observed excisions‡	Opportunities for excision§	Excision rate (%)	Observed excision‡	Opportunities for excision§	Excision rate (%)
FB	3	0	660	0.000	0	420	0.000
<i>copia</i>	2	0	440	0.000	0	280	0.000
<i>gypsy</i>	0	—	—	—	—	—	—
<i>flea</i>	2	0	440	0.000	0	280	0.000
<i>springer</i>	3	0	660	0.000	0	420	0.000
<i>opus</i>	2	0	440	0.000	0	280	0.000
<i>412</i>	6	0	1,320	0.000	0	840	0.000
<i>297</i>	8	1	1,760	0.057	0	1,120	0.000
<i>B104/roo</i>	13	0	2,860	0.000	0	1,820	0.000
<i>cDm2161</i>	9	0	1,980	0.000	0	1,260	0.000
<i>cDm2181</i>	0	—	—	—	—	—	—
<i>cDm2244</i>	0	—	—	—	—	—	—
<i>I</i>	0	—	—	—	—	—	—
<i>F</i>	6	0	1,320	0.000	0	840	0.000
<i>G</i>	0	—	—	—	—	—	—
<i>Doc</i>	6	0	1,320	0.000	0	840	0.000
<i>Delta 88</i>	1	0	220	0.000	0	140	0.000
<i>hobo</i>	17	0	3,740	0.000	0	2,380	0.000
<i>P</i>	11	9	2,420	0.372	0	1,540	0.000

* With the exceptions of the *copia*-like elements in *cDm2161*, *cDm2181*, and *cDm2244* (refs 17 and 18), the structures of the TE families studied are reviewed in ref. 19. The TE DNA clones used for *in situ* hybridization were as follows: FB (pD75.3), *copia* (cDm5002), *412* (cDm2042) and *297* (cDm4006) (gifts of G. M. Rubin)^{17,20,21}, *P* (p π 25.7BWC) and *Doc* (pw1.8) (gifts of K. O'Hare). *Flea* (fa*) *springer* (fa³), and *opus* (fa) were gifts of M. W. Young^{22,23}. *F* (pI 27F) and *G* (pI 17G) (gifts of P. Di Nocera). *I* (pI407) was a gift of D. Finnegan²⁴. *cDm2161*, *cDm2181* and *cDm2244* were gifts of C. Langley^{17,18}. *Delta 88* (tuh-st3) and *gypsy* (pgypBam) were gifts of W. Bender²⁵. *Hobo* (pH101) was a gift of S. Beckendorf²⁶. *B104/roo* (pACYCB104A) was a gift of C. Aquadro²⁷.

† We determined the initial sites for the elements *copia*, *I*, *hobo* and *P* directly from the *in situ* hybridizations of G₀ larvae. For each of the other TE families, we found that all seven nondysgenic lines had an identical set of sites at G₂₀. We therefore used that set as the probable G₀ sites.

‡ Excisions from the X chromosome were detected as *in situ* hybridization sites present at G₀ but not at G₂₀.

§ The no. of excision opportunities for each TE family was calculated by: (no. of X chromosomal sites) $\times$ (no. of generations) $\times$ (no. of lines).

|| The excision rate for each family was determined by dividing the observed number of excisions by the number of opportunities for excision.

Table 2 Insertion rates in dysgenic and nondysgenic lines

TE family*	Dysgenic lines				Nondysgenic lines			
	Total sites†	Observed insertions‡	Opportunities for insertion§	Rate (%)	Total sites†	Observed insertions‡	Opportunities for insertion§	Rate (%)
FB	30	1	6,600	0.015	31	0	4,340	0.000
<i>copia</i>	41	1	9,020	0.011	28	0	3,920	0.000
<i>gypsy</i>	3	0	660	0.000	2	0	280	0.000
<i>flea</i>	31	0	6,820	0.000	36	0	5,040	0.000
<i>springer</i>	15	0	3,300	0.000	13	0	1,820	0.000
<i>opus</i>	39	0	8,580	0.000	24	0	3,360	0.000
<i>412</i>	49	0	10,780	0.000	32	0	4,480	0.000
<i>297</i>	53	4	11,660	0.034	56	0	7,840	0.000
<i>B104/roo</i>	113	1	24,860	0.004	83	1	11,620	0.009
<i>cDm2161</i>	58	0	12,760	0.000	49	0	6,860	0.000
<i>cDm2181</i>	36	0	7,920	0.000	36	0	5,040	0.000
<i>cDm2244</i>	47	0	10,340	0.000	42	0	5,880	0.000
<i>I</i>	27	0	5,940	0.000	30	0	4,200	0.000
<i>F</i>	66	0	14,520	0.000	60	0	8,400	0.000
<i>G</i> ¶	0	0	0	—	0	0	0	—
<i>Doc</i>	48	0	10,560	0.000	48	0	6,720	0.000
<i>Delta 88</i>	12	0	2,640	0.000	11	0	1,540	0.000
<i>hobo</i>	41	0	9,020	0.000	65	0	9,100	0.000
<i>P</i>	31	193	6,820	2.830	50	4	7,000	0.057

* The DNA clones used for *in situ* hybridization are listed in Table 1. † The G₀ sites were determined as in Table 1.

‡ Insertions were detected as *in situ* hybridization sites absent on the X chromosome at G₀ but present at G₂₀.

§ The number of insertion opportunities for each TE family was calculated by multiplying the quantities: (total number of euchromatic sites) $\times$ (number of generations) $\times$ (number of lines). The total number of elements present at each generation was calculated by adding the number of sites initially present on the *sn*^w X chromosome, to the number of euchromatic autosomal sites observed. For nondysgenic lines, the number of autosomal sites was estimated as twice the average number of autosomal *in situ* hybridization sites observed in 5–10 nuclei from each of two larvae from the C(1)DX, *yf*; π_2 stock. For dysgenic lines, the number of autosomal elements in the genotype *sn*^w; *bw*/ π_2 ; *st*/ π_2 was calculated by adding the haploid number of autosomal sites present in the *sn*^w; π_2 stock to the haploid number of autosomal sites in C(1)DX, *yf*; *bw*; *st*. The numbers of autosomal *in situ* hybridization sites in these two stocks were determined as described above for C(1)DX, *yf*; π_2 .

|| The insertion rate for each family was determined by dividing number of observed X chromosomal insertions by number of insertion opportunities.

¶ No euchromatic sites were detected for the *G* family although heterochromatic copies were inferred from *in situ* hybridization labelling over the chromocentre. The data were therefore included because transpositions from the heterochromatin to the X chromosome were possible.

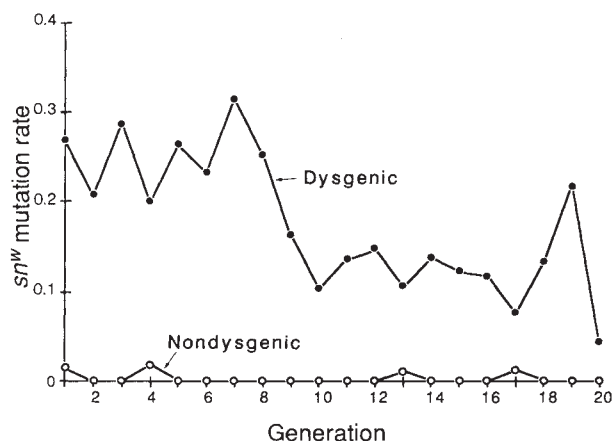


Fig. 2 The average sn^w mutability for dysgenic lines (●) and for nondysgenic lines (○) is plotted for each generation. The total number of flies scored each generation ranged from 363 to 1,046 (averaging 575) for dysgenic lines, and from 285 to 1,006 (averaging 495) for nondysgenic lines.

More recently Woodruff *et al.*¹⁴ measured the reversion rates of transposable element insertion mutations at visible loci. They concluded that P-M hybrid dysgenesis did not enhance reversions of non-P TE mutations. Their assay, however, did not address transposition (as opposed to excision) events, nor could it have detected imprecise excisions that failed to induce phenotypic changes. Thus, considerable cross-mobilization of non-P elements could have gone undetected. Similarly, W. Eanes (personal communication) found no evidence of cross-mobilization using *in situ* hybridization. His sample size, however, was too small to support a general conclusion for the lack of mobility.

We have avoided the above problems by means of a crossing procedure in which all tested X chromosomes were derived from a single starting chromosome without recombination or segregation. The initial distribution of each transposable element family was determined for this chromosome. The use of nondysgenic controls and the continuous monitoring of sn^w instability allowed us to relate any observed activity of TE families specifically to P-M hybrid dysgenesis. Our detection methods also allowed us to observe both transposition and excision events without requiring that such events result in gross phenotypic changes. We agree with the conclusion of Woodruff *et al.*¹⁴ that P-M hybrid dysgenesis does not result in a measurable increase in the activity of other TE families.

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Acknowledgements should be brief. Grant numbers and contribution numbers are not allowed.

Transverse alternating electrophoresis

K. Gardiner and D. Patterson

Pulsed field gel techniques have pushed the size limits for the separation of DNA fragments forward by several orders of magnitude. By orienting the electric field transverse to the gel, lane-to-lane distortion of DNA migration can be eliminated.

CONVENTIONAL gel electrophoresis for the separation of DNA uses an electric field that is constant in time, and is uniform across the width of the gel. For practical purposes, the upper limit on the size of DNA molecules that can be resolved by this method is approximately 50,000 base pairs¹. Pulsed field gel electrophoresis uses electric fields which alternate direction at specified time intervals throughout the gel run^{2,4}. This regular alternation of field orientation, first demonstrated by Schwartz and Cantor⁵, allows resolution of very large DNA molecules. At present, with appropriate electrode geometry and running conditions, molecules as large as nine million base pairs can be separated. No definitive theory has yet been developed to explain the increased resolution of pulsed field gels, however, so there is no reason to postulate this as an upper limit.

The first pulsed field designs were shown to be capable of separating into distinct bands the chromosomes of the yeast, *Saccharomyces cerevisiae*, and those of several species of unicellular parasites^{2,3,6}. However, these early designs used horizontal gels and electrodes that generated fields which were not uniform across the gel. This resulted in marked variation of the migration paths of the DNA from lane to lane in response to the different field patterns. Not only did the DNA not migrate straight down the gel but, in many cases, the distortions in migration were sufficient to make size comparisons between samples difficult. Several more recent designs have corrected this deficiency by adding more electrodes, increasing the complexity of the switching routines, re-orienting the gel during electrophoresis, or sacrificing the resolution of larger molecules⁷⁻¹⁰. Here we describe a vertical pulsed field gel system that is not only simple in design and easy to use, but also gives perfectly straight and equivalent migration of DNA down the gel from lane to lane throughout the gel.

The transverse angle

A sketch of the current vertical pulsed field gel apparatus is shown in Fig. 1. It is longer than the original model⁴, but simpler in design. It consists of a fish tank-like plexiglass box, with two pairs of electrodes strung horizontally across the width. The gel stands vertically at the centre of the box and is supported by two thin slots at either edge, and by the buoyancy of the buffer which fills the box. Dur-

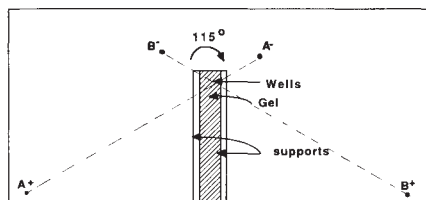


Fig. 1 Schematic representation of the vertical pulsed field electrophoresis apparatus. The electrode pairs A (+ and -) and B (+ and -), shown as points, are wires stretched across the width of the box parallel to the gel faces. The angle formed at the sample loading wells between the fields generated by the A and B electrodes is 115°.

ing electrophoresis, the A electrodes (+ and -) are activated together, and alternate with the B electrodes. Thus, when A+ and A- are activated, the resultant electric field passes downward and through the face of the gel, forcing the DNA downward and to the left. If the A electrodes are then turned off, and the B electrodes activated, the DNA is pushed downward and to the right. Hence, the name "transverse alternating field". It is this field geometry, transverse to the plane of the gel rather than within the plane of the gel, that is responsible for the uniform migration of DNA regardless of the position of the lane. Because all electrodes are parallel to the face of the gel, all lanes experience identical electric fields and there is no net lateral force within the plane of the gel.

Figure 2 shows an ethidium bromide stain of a typical gel run, illustrating the uniformity of the DNA migration. Lanes 1, 5 and 9 contain preparations of *S. cerevisiae* chromosomes. The band patterns are clearly identical, making the determination of corresponding bands in each lane unambiguous. The remaining lanes contain mammalian DNAs which have been digested with the restriction enzymes *Bss*HII and *Sfi*I. The uniform migration of DNA from one lane to another is a feature which is particularly important for studies of mammalian genomes, whose large chromosomes must be digested with endonucleases before electrophoresis. The ability to compare and interpret the hybridization patterns in all of the lanes of a gel in a straightforward manner is critical in the search for chromosomal breakpoints, as well as in other studies of mammalian gene organization.

Also noteworthy in Fig. 2 are the light

and dark band patterns in the ethidium-stained mammalian DNA. These are observed routinely in highly purified preparations of completely digested DNA, and are unique to each enzyme and mammalian DNA combination. (Compare the hamster DNA in lane 2 with the human DNAs in lanes 3 and 4.) It is not yet clear what significance, if any, these band patterns may have.

Resolution

The running conditions used for the gel in Fig. 2 were chosen because they yield good overall resolution of relatively large pieces of DNA. The pulse time (the frequency of switching between electrode pairs A and B) was 50 sec at 13 V cm⁻¹, for 18 hours. The largest yeast band visible is approximately 1,500 kilobases and clearly there is resolution of significantly larger fragments of mammalian DNA. The smallest material seen is approximately 30 kilobases. As with all pulsed field gel systems, it is largely the pulse time that dictates the size class to be resolved. For example, short pulse times of between two and four seconds are effective for examining molecules in the range of approxi-

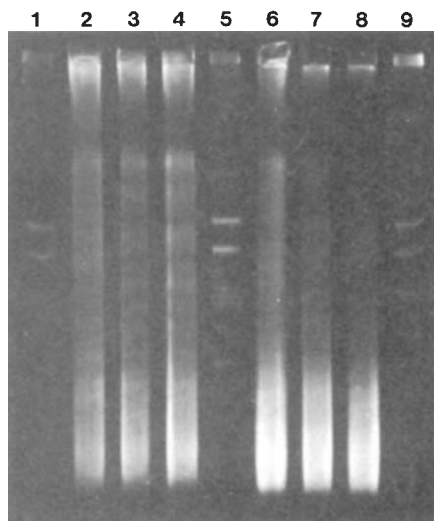
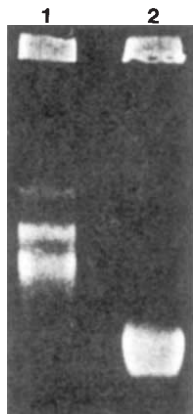


Fig. 2 Ethidium bromide stained gel showing resolution of yeast and mammalian DNA. Lanes 1, 5 and 9 are *S. cerevisiae* DNA preparations; each band represents a yeast chromosome. Lanes 2 and 6 are Chinese hamster DNA, digested with *Bss*HII and *Sfi*I, respectively. Lanes 3 and 4, and 7 and 8, are human DNAs digested with *Bss*HII and *Sfi*I, respectively. The gel is 0.8 per cent agarose, run for 18 hours with a 50 sec pulse time, starting at 250 V. The largest yeast band is approximately 1,500 kilobases, the smallest, 200 kilobases.

Fig. 3 Ethidium bromide stained gel illustrating the range of resolution of transverse alternating field electrophoresis. Lane 1 is DNA prepared from the three chromosomes of the fission yeast *S. pombe*. The bands shown are three, six and nine million base pairs. Lane 2 is DNA isolated from *S. cerevisiae*, which is not resolved under these conditions.



mately one kilobase to 150 kilobases, and 30 seconds maximizes the resolution of the 50 to 500 kilobase size class.

Figure 3 illustrates the maximum size of DNA that can be resolved at present using transverse alternating field electrophoresis. The three chromosomes of the fission yeast *S. pombe* are thought to contain roughly three, six and nine million base pairs of DNA, respectively^{11,12}. By dramatically altering the running conditions to a 90-minute pulse time at 1.5 V cm⁻¹ for four days, the DNA from these chromosomes can be separated as shown. That each band represents a single, discrete chromosome has been verified by hybridization using chromosome-specific DNA probes (unpublished data). Increasing the angle between the electrodes may allow the resolution of the smallest human chromosomes, at approximately 50 million base pairs.

The vertical pulsed field system shown here has been used for the identification of chromosome number in mammalian parasites, gene mapping and studies of chromosomal organization in organisms from bacteria to man, and the study of irradiation-induced DNA damage in yeasts and mammals. Equipment for performing transverse alternating field electrophoresis is now commercially available from Beckman Instruments, Inc. □

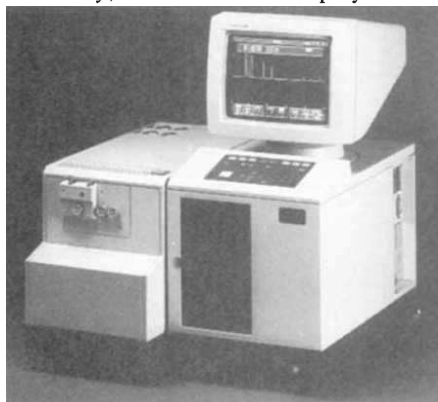
Katheleen Gardiner and David Patterson are at the Eleanor Roosevelt Institute for Cancer Research, 1899 Gaylord Street, Denver, Colorado 80206, USA. For more information, fill in reader service number 100.

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Separation and analysis

Chromatography and electrophoresis form the backbone of modern biology. Described below are products suited for applications from the determination of molecular weights to the analysis of neurotransmitters.

THE newest **gas chromatograph** from the Perkin-Elmer 8000 series is the model 8700, a dual-channel instrument with a full-colour monitor (*Reader Service No. 101*). The \$18,000 (US) model 8700's two channels can be operated singly or simultaneously, with the results displayed on a



The Perkin-Elmer 8700 gas chromatograph.

split or overlaid screen, in real time or after the run. The instrument's software can perform multi-level calibration and independent integration of both channels, and can handle multiple reference peaks with re-integration facilities. The model 8700 gas chromatograph compares sample and reference chromatograms graphically, and can be connected to a computer for the transfer of methods, raw and re-integrated data, and reports.

The TLC Scanner II from Camag performs quantitative evaluation of **thin-layer chromatograms** from fluorescence or absorbance, using a Hewlett-Packard



A fully-configured **TLC Scanner II** from Camag. 310 laboratory data system (*Reader Service No. 102*). The parameters for TLC evaluation can be set before each run by responding to queries from the computer, or be recalled from computer memory for automatic routine operation. After scanning begins, the TLC Scanner II automatically locates and optimizes the fraction zones, according to four selectable modes. The system's evaluation software computes the results by peak height and

peak area, and displays both intermediate and final results so the plausibility of results can be checked during the run. Camag's scanner system has a dual wavelength scanning function to eliminate background irregularities, and automatic baseline correction and structure subtraction. Problematic chromatograms can be integrated on the video screen.

A **timed-split injection assembly** for capillary supercritical fluid chromatography (SFC) is available from Lee Scientific (*Reader Service No. 103*). Timed-split injection in capillary SFC eliminates the need to use packed columns, and allows the technique to be used in trace analysis. Lee Scientific says the use of a timed-split injector permits the introduction of samples ranging in volume from 1 nl to 0.5 µl onto capillary columns, with results that are more representative of the sample composition than with conventional split injection techniques.

Cuno offers a complete line of products for **affinity chromatography**, under its Zetafinity brand (*Reader Service No.*

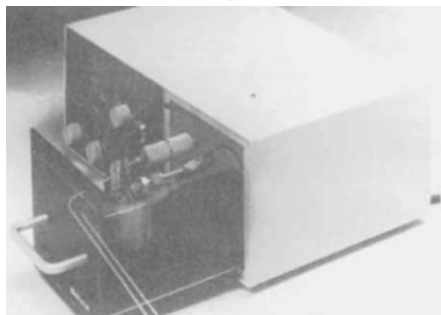


Cuno's Zetafinity affinity chromatography line.

104). The Zetafinity products have a cellulose-polymer support that Cuno says offers very high flow rates with no channelling. The ligand of choice can be immobilized on Zetafinity columns at neutral pH, without the use of toxic substances. Zetafinity columns come in sizes suited for methods development, lab sampling and pilot and production plant needs, and they can be regenerated and sterilized. Three different chemistries are available: amino, hydroxyl, and pre-activated amino in aldehyde form for faster coupling of ligands.

The German company Biometra has developed a system for the measurement

of acetylcholine and choline by **enzyme-linked HPLC** (*Reader Service No. 105*). In the Biometra system, acetylcholine and choline are first separated by HPLC,



Enzyme reaction coupled with HPLC from Biometra.

and then flow into an enzyme reactor where acetylcholine is hydrolysed to choline and acetate, and choline is oxidized to betaine and H_2O_2 . The H_2O_2 end-product is then measured by an electrochemical detector, allowing the amount of acetylcholine to be calculated. Biometra says the system can detect down to 0.1 pmol of choline or acetylcholine within a sample of 20 μ l, and that the covalent link between the matrix and enzymes in the reactor keeps the reactor active for several months of continuous use.

Electrophoresis

A **calibration kit** for electrophoresis of high molecular weight-SDS compounds is available from Pharmacia (*Reader Service No. 106*). The kit contains 10 vials of five

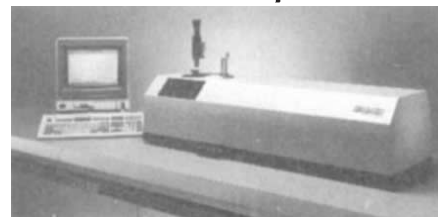


Pharmacia's kit for calibrating electrophoretic gels.

lyophilized protein standards that cover the range of 53,000–212,000 daltons. Reconstitution of the vial contents in 100 μ l of water provides a protein solution dissolved in sample buffer. Pharmacia says the sample buffer results in fully denatured protein standards, enabling direct application of the vial contents to an electrophoresis gel without heating. Each vial also contains bromophenol blue to facilitate sample application and monitoring of run progress, and 3 per cent D-mannitol to enable direct application on vertical electrophoresis gels.

Precast polyacrylamide gels in both single concentration and gradient formats are available from Novex (*Reader Service No. 107*). The 8 $\times$ 8 cm gels are supplied in disposable, thermally sealed plastic cassettes, complete with combs and preformed sample wells. For use with the gels, Novex also offers a dual-slab electrophoresis apparatus with a simple wedge clamp to seal the gel cassettes in the buffer chamber, and a Western blot transfer module which requires only 100 ml of buffer. Novex says the module transfers typical proteins in one hour or less.

The DELSA 440 from Coulter Electronics is a **laser-based particle electro-**



phoresis analyser that measures particle electrophoretic mobility, distribution, zeta potential, average particle size and the specific conductance of charged particles in liquid suspensions, under the control of an IBM computer (*Reader Service No. 108*). It makes independent measurements at four different angles of laser light simultaneously, a feature that allows the detection of different sizes of

particles in a sample, even though they have similar surface charges and mobilities. The \$65,000 (US) DELSA 440's software is based on Microsoft's Windows, and the data are presented in colour. The DELSA 440 can analyse particles ranging in size from 10 nm to 20 μ m, and Coulter says the system can measure the electrophoretic mobility of any charged particle from coal dust to membrane vesicles and lymphocytes in under 5 minutes.

Shimadzu has a multi-purpose **densitometer** that can be used with thin-layer chromatography, electrophoresis, autoradiography and fluorescence applica-

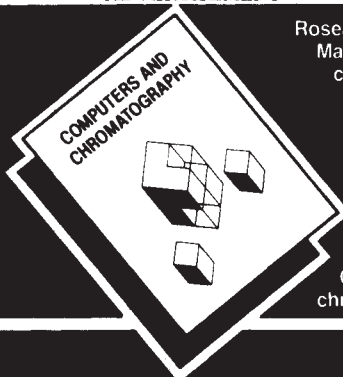


Shimadzu's densitometer for 2-D electrophoresis. (*Reader Service No. 109*). The model CS9000 densitometer has a flying-spot zig-zag scanning capability that enables it to perform 2-dimensional contour mapping of the sample area. The basic instrument includes a colour CRT, high-resolution printer/plotter and a monochromator that can be set for wavelengths between 200 and 700 nm. The double optical system of Shimadzu's densitometer uses three photomultipliers with one as a reference, for better reproducibility of results. To separate close bands and TLC spots, resolution down to 50 μ m can be selected, and chromatograms can be reintegrated on the system's CRT.

Biocent, from the Netherlands, sells equipment for **field inversion gel electrophoresis** (*Reader Service No. 110*). Biocent's GENE-TIC system is a modular set of devices that can be used to convert standard polyacrylamide gel electrophoresis apparatus into a set-up for field inversion gel electrophoresis. The four-

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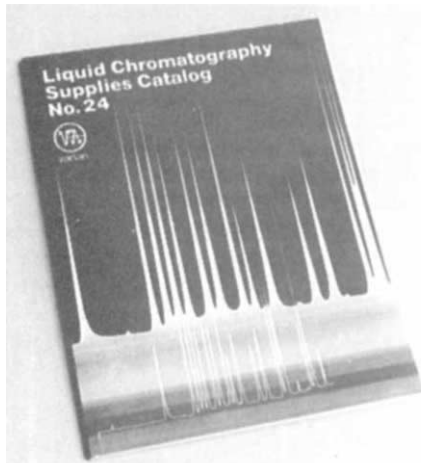
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channel GENE-TIC power supply and switching device come with necessary software, and can be used with any IBM-compatible PC.

Liquid chromatography

Varian has a new liquid chromatography **supplies catalogue** featuring columns and supplies for Varian's line of liquid



The book on liquid chromatography from Varian.

chromatographs (*Reader Service No. 111*). The free catalogue contains two expanded sections: one on columns and packings and the one on sample preparation. Another chapter is a guide to selecting the correct supplies. Other sections list valuable books and manuals, supplies for solvent purification, laboratory accessories and data handling. The text is illustrated with graphs, spectra and technical notes providing detailed applications for various columns and advanced automated sample preparation.

ChromNet is the name of a new liquid chromatography **system controller** from Spectra-Physics (*Reader Service No. 112*). ChromNet sets up and monitors methods



ChromNet from Spectra-Physics controls multiple systems operating on a local area network.

and analyses for multiple systems operating on LABNET, Spectra-Physics' local area network. The \$950 (US) controller is an IBM-compatible menu-driven program with setup screens that prompt for

parameter entries, system configuration, analysis description and method information. Both methods and analyses can be stored in memory for later use. The system performs communications checks to ensure that all modules are operating correctly, performs error checking to see that parameter entries are within acceptable ranges, and gives an ongoing status report on-screen. Context-sensitive help screens are available at any time by pressing one key.

HPLC

A programmable UV-visible monitor is available from Bio-Rad that offers continuously selectable wavelength capability, without the need to change the lamp or filter (*Reader Service No. 113*). The model 1706 has keypad input and microprocessor control, and a time program mode that allows initial values of wavelength and range to be changed at four times selected by the user. The monitor has high sensitivity, combined with an 8 nm band pass for measurement at all wavelengths without background interference. The LCD display shows values for absorbance, range, wavelength, time-constant response, lamp energy, sample side energy, detector electronics and detector diagnosis. Self-diagnostics verify major instrument functions.

Gilson Medical Electronics will soon be introducing a new variable-wavelength **UV detector** that offers detection from 190 to 380 nm (*Reader Service No. 114*). The model 115 UV detector is designed to fit into a Gilson HPLC stack, but can also be used with other commercially-available systems. The detector has a sensitivity range of 0.0001-1.0 AUFS and a 1 per cent typical peak-to-peak noise level at 0.0001 AUFS, says Gilson. Six different flow cells ensures linear detection and a stable baseline over a broad dynamic range. Event marks and autozero functions can be initiated using buttons on the front panel or through remote contact closure. Three outputs from the rear panel (10 mV variable, 100 mV variable and 1 V fixed) send detector output to a chart recorder and to a data analysis system.

An **HPLC system optimized for mass spectroscopy** has been introduced by the Waters division of Millipore (*Reader Service No. 115*). The \$17,000 (US) model 600MS solvent delivery module is a high-performance gradient pump with four-solvent capability. It has software and fluidics that confer compositional accuracy and precision for reproducibility of results. Waters says the optimized hydraulic system eliminates pump pulsation for precise, high-sensitivity MS quantitation. The \$10,500 (US) model 490MS absorbance detector features a high-pressure flow cell to accommodate

4000 p.s.i. backpressure, allowing plumbing before the MS interface.

Plug-in packs of **databases for chromatography** are available from Phase Separations Ltd (*Reader Service No. 116*). The hand-held Psion Organiser II holds databases of up to 128,000 words of data, abstracted from all the relevant publications. A find button simplifies searches, without the need to dial up electronic net-



No entry codes are required to tap chromatography databases with the Phase Separations Psion II.

work databases and enter passwords. The Psion Organiser II also has a built-in calculator, programmable computer, diary, alarm clock and calendar.

Pilot- and production-scale chromatography is possible with HPLC systems from Prochrom (*Reader Service No. 117*). The Series LC100 system has a column efficiency of up to 75,000 theoretical plates per meter and a throughput of 130 g per injection, says Prochrom. The system uses axial compression to achieve high efficiency in a column 11 cm in diameter and up to 60 cm in length. The system can be operated manually or automatically with automatic sample injection, four-solvent gradient, effluent collection, recycling mode, guard column, backflush, alternate mode and on-line analytical sampling. The columns for the Series LC100 are packed by the user on-site. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal and apply only within the country indicated.